TITLE

REPRODUCTION AND COAGULATION FACTOR XIII IN WOMEN

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DECLARATION

I DECLARE THAT THE WORK PRESENTED IN THIS THESIS IS MY OWN.

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ABSTRACT

Factor XIII (FXIII) deficiency is a rare bleeding disorder with an average frequency of one case in 1-3 million and has an autosomal recessive inheritance. Previous case reports and case series have demonstrated an increase in the risk of miscarriage and placental abruption among women with FXIII deficiency. However, there are limited data on the borderline level of FXIII that is needed to avoid obstetric complications.

The aim of this study is to obtain a better understanding about the clinical course and outcome of FXIII deficiency among women.

A systematic review of literature has been performed for congenital FXIII deficiency among women and its associated reproductive outcomes using an electronic search on databases. A total of 116 women were identified from 34 articles. From these cases, it was concluded that women with congenital FXIII deficiency suffer significant bleeding complications. Menorrhagia and ovulation bleeding are common gynaecological problems and possibly more prevalent than reported. Pregnancies in women with FXIII deficiency have a significant risk of miscarriage, placental abruption, preterm delivery and PPH if not treated.

Pregnancy and the early post delivery period can be associated with alteration in FXIII activity. To establish the reference range for FXIII level during pregnancy and immediate puerperium, a cross sectional study of 376 healthy pregnant women was conducted to measure FXIII activity during first (weeks 0-12, n=116), second (weeks13-28, n=132) third trimester (weeks 29-42, n=128) and postnatal period (day0-3; n=30). A longitudinal study on 26 women from the same population was also performed throughout their period of

gestation. FXIII activity measured during the menstrual cycle of non-pregnant women (n=25) was used as a control group. There was a statistically significant reduction in the FXIII activity in the second and third trimester compared to the first trimester of pregnancy ($p<0.0001$). Mean FXIII level during second and third trimester and postnatal period were significantly lower compared to control group.

A second study was also performed involving 32 women of reproductive age to assess the level of FXIII during various phases of menstrual cycle, aiming to examine possible changes in plasma FXIII activity during the normal menstrual cycle and whether FXIII activity during the menstrual phase can be correlated with heavy menstrual loss based on pictorial blood-assessment chart (PBAC).

FXIII level was higher during periovulatory and secretory phases of the cycle compared to the menstrual phase and this difference was found to be statistically significant ($p=0.036$). No significant correlation was found between FXIII level during menstrual phase and BPAC score ≥ 100.

The third study evaluates FXIII activity among 68 women with recurrent miscarriage, compared to 62 women with no history of pregnancy loss and at least one living child. Even though women with history of recurrent miscarriages had lower FXIII activity compare to the control group, such difference did not reach a statistical significant level ($p=0.142$). However, the cohort of women with history of miscarriage had significantly more cases with FXIII activity <70 IU/dL compared to the control group ($p=0.034$).

In conclusion, women with congenital FXIII deficiency suffer significant obstetrics and gynaecological complications if not treated. Pregnancy is associated with a significant decrease in FXIII activity, reaching the lowest level during the third trimester of pregnancy. FXIII activity was also found to be lowest during menstrual and preovulatory phase of the cycle. Women with history of recurrent miscarriage are more likely to have low FXIII (<70 IU/dL) levels.

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ABBREVIATIONS

ANOVA	Analysis of Variance
APH	Antepartum haemorrhage
APTT	Activated partial thromboplastin time
BAT	Bleeding assessment tool
BMI	Body Mass Index
cFXIII	Cellular form of Factor XIII
CS	Caesarean section
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and drug agency
FFP	Fresh frozen plasma
FSF	Fibrin Stabilising Factor
FSH	Follicle-stimulating hormone
FXIII	Factor XIII
FXIIIa	Active transglutaminase
FXIII-A	Factor XIII A subunit
FXIII-B	Factor XIII B subunit
HMB	Heavy menstrual bleeding
IBD	Inherited bleeding disorders
IFCC	International Federation of Clinical Chemistry
ICH	Intracranial haemorrhage
ISTH-SSC	International society of Thrombosis and Haemophilia – Scientific and Standardization Committee
IVF	In vitro fertilisation
IU	International Unit

IUD	Intrauterine device
IUGR	Intrauterine growth restriction
LH	Luteinizing hormone
OCP	Oral Contraceptive Pills
PBAC	Pictorial blood-assessment chart
PAI-1	Plasminogen activator inhibitor type 1
PPH	Postpartum haemorrhage
PPP	Platelet poor plasma
PT	Prothrombin time
RFH	Royal Free Hospital
SD	Standard Deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TSP-1	Thrombospondin-1
UKHCDO	United Kingdom Haemophilia Centre Doctors' Organisation
UK NEQAS	United Kingdom National External Quality Assessment Scheme
VWF	Von Willebrand Factor
VWD	Von Willebrand Disease

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Inherited bleeding disorders in older women
Rezan A. Kadir, Lava A. Sharief, Christine A. Lee. *Maturitas*. 2012 Vol. 72(1): 35-41.

Congenital Factor XIII deficiency in women; a systematic review of literature
Lava Sharief, Rezan Kadir. *Haemophilia*. 2013 Aug 28

Changes in FXIII level during various stages of pregnancy
Lava Sharief, A.S. Lawrie, I.J. Mackie, C. Smith, F. Peyvandi , R.A. Kadir , *Haemophilia* Journal, In press.

Plasma FXIII variation during menstrual cycle
Lava Sharief, A.S. Lawrie, I.J. Mackie, S. Halimeh, C. Smith, F. Peyvandi, R.A. Kadir , *Haemophilia* Journal, In press.

POSTER PRESENTATIONS

Congenital Factor XIII deficiency in women; a systematic review of literature
Lava Sharief, Rezan Kadir, , **I**STH XXIV congress, Amsterdam, July 2013.

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CHAPTER ONE

INTRODUCTION

1 CHAPTER ONE: INTRODUCTION

1.1 General Introduction

Factor XIII (FXIII) deficiency is a rare hereditary autosomal recessive bleeding disorder with an average frequency of one in 1-2 million (Peyvandi et al., 2009). There is a higher incidence of this condition in parts of the world that have a high rate of consanguinity (Bolton Maggs et al., 2004). Congenital FXIII deficiency is characterised by severe, delayed, spontaneous bleeding with normal coagulation screening tests.

Women are more vulnerable to manifest a bleeding disorder because they are at high risk to experience bleeding challenges in their lifetime as a result of the natural cyclical bleeding during monthly menstrual and ovulation, as well as child birth. Women with FXIII deficiency often fail to produce the same levels of clotting factors as normal women, making them vulnerable to bleeding diathesis, delayed wound healing and adverse pregnancy outcome, mainly miscarriage.

Women with congenital FXIII deficiency have been known to be a risk factor for recurrent early pregnancy loss (Rodeghiero et al., 1987; Inbal and Kenet, 2003; Asahina et al., 2007). Bleeding complications in relation to pregnancy, namely antepartum and postpartum haemorrhage have also been observed in women with FXIII deficiency (Saito et al., 1990; Mikkola et al., 1997); however, their prevalence is not clear. Postpartum haemorrhage have been observed in few case reports and series of women with FXIII deficiency (Saito et al., 1990; Burrows et al., 2000; Ivaskevicius et al., 2010b). Haemorrhagic ovarian cysts and menorrhagia were also observed in women with this factor deficiency. In one case series involving 20 women with FXIII deficiency, menorrhagia occurred in 35% of cases, while

20% experienced ovarian bleeding, requiring hysterectomy in one of the cases (Lak et al., 2003).

Different guidelines provide different recommendations regarding the dose and interval of FXIII concentrate during pregnancy as well as the level above which FXIII should be maintained to avoid adverse pregnancy outcome (Rodeghiero et al., 1987; Boda et al., 1989; Asahina et al., 2000, 2007; Burrows et al., 2000). In addition, reference intervals for FXIII activity during each trimester of normal pregnancy have not been established yet. There is also lack of data in literature on the role of FXIII level in women with unexplained recurrent miscarriage.

1.2 Aims and Objectives

This thesis aims to assess obstetric and gynaecological complications for women with congenital FXIII deficiency. In addition, the thesis addresses the effect of menstrual cycle and normal pregnancy on FXIII level. Considering the link between congenital severe FXIII deficiency and risk of recurrent pregnancy loss, in this thesis FXIII level was assessed in women with unexplained recurrent miscarriage to determine whether FXIII level is altered in this group of women and whether borderline levels of FXIII is also associated with risk of miscarriage.

The layout of the thesis is as follows;

- Chapter two provides an overview of FXIII, its structure, and function. In addition, it also provides a thorough review of FXIII deficiency, its clinical presentation, diagnosis and management in general.
- Chapter three provides a systematic review of the literature of case reports and case series of women with congenital FXIII deficiency. The review focuses on obstetric complications and outcome as well as of gynaecological problems in women with FXIII deficiency. The review provides data on the frequency and severity of menstrual problems, in particular, heavy menstrual bleeding and ovulation bleeding. It also review literatures on the pregnancy complications, mainly risk of miscarriage and bleeding during pregnancy and postpartum.
- Chapter four is a study of the FXIII changes during pregnancy and postnatal period. The aim is to establish the reference ranges of FXIII level during each

trimester of pregnancy and early postnatal period and to assess changes in FXIII level in normal uncomplicated pregnancy.

- Chapter five is a study on the variation of the FXIII level during the menstrual cycle. The objective is to measure and compare plasma FXIII activity during menstrual, late follicular, periovulatory, secretory and premenstrual phases of the cycle and to assess any correlation between FXIII level and menstrual blood loss.
- Chapter six assesses the role of FXIII level in women with recurrent miscarriages. FXIII activity level is measured in a group of women with history of recurrent miscarriage and compared it to the levels of a control group of women with no history of miscarriage and one living child. The study aimed to assess any possible association between borderline plasma FXIII level and the risk of recurrent miscarriage.

CHAPTER TWO

BACKGROUND AND LITERATURE REVIEW

2 CHAPTER TWO: BACKGROUND AND LITERATURE REVIEW

2.1 Historical background:

In 1944, Robbins *et al* described the formation of insoluble fibrin when there was a combination of calcium with an unknown serum factor. This finding was further supported in 1948 by Laki and Lorand who concluded that the factor was a plasma protein known as Fibrin Stabilizing Factor (FSF) (Robbins, 1944; Laki and Lorand, 1948). FSF was later given into different synonyms including Laki–Lorand or L–L factor, fibrinase, protransglutaminase and fibrin polymerase. In 1963, the International Committee of Blood Clotting Factors used the nomenclature of factor XIII as a unified abbreviation for this coagulation factor (Muszbek et al., 2007).

Duckert *et al* (Duckert et al., 1960) published the first case report of FXIII deficiency in 1960 in a 7 year old boy from Switzerland with prolonged bleeding following a sharp cut on his forehead. His previous bleeding history included umbilical bleeding at birth as well as a large haematoma on the head and subdural haematoma 24–36 hours following minor trauma. He also had slow poor wound healing. His parents were consanguineous and he had five siblings, of whom one brother had similar bleeding symptoms. However, his parents and four other siblings had no bleeding tendency. His bleeding was described as being due to deficiency of FXIII based on his delayed bleeding episodes, abnormal clot solubility assay, and thromboelastography.

The thromboelastography test revealed a decrease in the maximal amplitude and a fast decline in clot size and strength, while the clot solubility assay showed an increased breakdown of clots in 5 M urea. Fresh Frozen Plasma (FFP) was used to treat the persistent forehead bleeding and the clot solubility test returned to normal (Duckert et al., 1960; Schroeder et al., 2007).

2.2 Factor XIII Structure:

Plasma FXIII is a zymogen of tetrameric structure with two potentially active/catalytic A-subunits (FXIII-A) and two carrier/noncatalytic B subunits (FXIII-B), with a total molecular weight of 320 kDa. The average concentration of plasma FXIII (A2B2) is 14-28 mg/L^{-1}, corresponding to 67-133 IU/dL (Katona et al., 2000). FXIII-A subunits in plasma is present only in complex form, while 50% of FXIII-B subunits is available in the free, non-complex form. Patients with heterozygous and homozygous FXIII deficiency are characterised by a reduction in the total level of the B subunit, while the free B subunit remains constant (Yorifuji et al., 1988). A cellular form of factor XIII (cFXIII) has been identified in platelets and monocytes/macrophages. This form exists as a homodimer of two A-subunits with a total molecular weight of 166 kDa (Muszbek et al., 1996).

FXIII-A contains both the active thrombin cleaving site and the calcium-binding site required for catalytic activation (McDonagh, 1994). The gene coding for the FXIII-A subunit (Figure 2.1) is located on chromosome 6p24–25, and consists of 15 exons separated by 14 introns, which all together span over 160 kb and encoding a mature protein of 731 amino acids. The three- dimensional X-ray crystallographic structure of recombinant FXIII-A subunit showed that the A subunit folds into distinct domains that are arranged in the following sequence: the N-terminus activation peptide at residues 1-37, the beta sandwich at residues 38-183, the catalytic core region at residues 184-515, beta barrel 1 at residues 516-627, and beta barrel 2 at residues 628-730 (Yee, 1994, 1996). Among the nine cysteine residues in the protein, cysteine 314 represents the active site, but none of them form disulfide bonds. The catalytic mechanism of cross-linking by transglutaminases requires the catalytic triad in the central core domain which is formed through hydrogen bond interactions between Cys314, His373 and Asp396 (Pedersen, 1994). Two non-proline cis peptide bonds between Arg310 and Tyr311, and Gln425 and Phe426 have also been proposed to be important for FXIII function (Weiss et al., 1998).

The B subunit of FXIII (FXIII-B) is a mosaic protein composed of 10 tandem repeats, called glycoprotein-1 structures or Sushi domains because of their shape. The FXIII-B subunit gene is located on chromosome 1q31–32.1, composed of 12 exons interrupted by 11 introns and spans approximately 28 kb and is encoding the mature protein of 641 amino acids (Figure 2.2) (Bottenus et al., 1990).

The B subunit is important in the process of stabilisation and transport of the hydrophobic A2 subunit in the aqueous environment of human plasma, thereby prolonging FXIII-A2 in circulation. Another important role is initiating the cross-linking process through localizing FXIII to the growing fibrin polymer, while the thrombin is still active. Localisation is facilitated when FXIII-B portion of the Factor XIII A2B2 molecule binds specifically to the gamma chains of fibrinogen leading to polymerisation, cross-linking and regulation of FXIII activity (Siebenlist et al., 1996; Mosesson, 2003).

The primary formation of FXIII-A is from haematopoietic cells, while the hepatocytes appear to be responsible for the production of FXIII-B. The origin of FXIII subunits was identified from studies of hepatic and bone marrow transplantation, since recipients of a liver transplant experiences a change in their FXIII-B phenotype to the donor's phenotype, without a similar change in FXIII-A phenotype. Likewise, FXIII-A phenotype would convert to the donor’s phenotype only after bone marrow transplantation (Wölpl et al., 1987). The intracellular FXIII found in megakaryocytes and platelets is in the form of two A-subunits, while B-subunits are absent, with circulating platelets containing around 50% of the total FXIII found in whole blood. Cellular FXIII-A have the same immunochemical structure of plasma FXIII-A and thus can combine with FXIII-B to form the tetramer complex when released into plasma (Hsieh and Nugent, 2008).

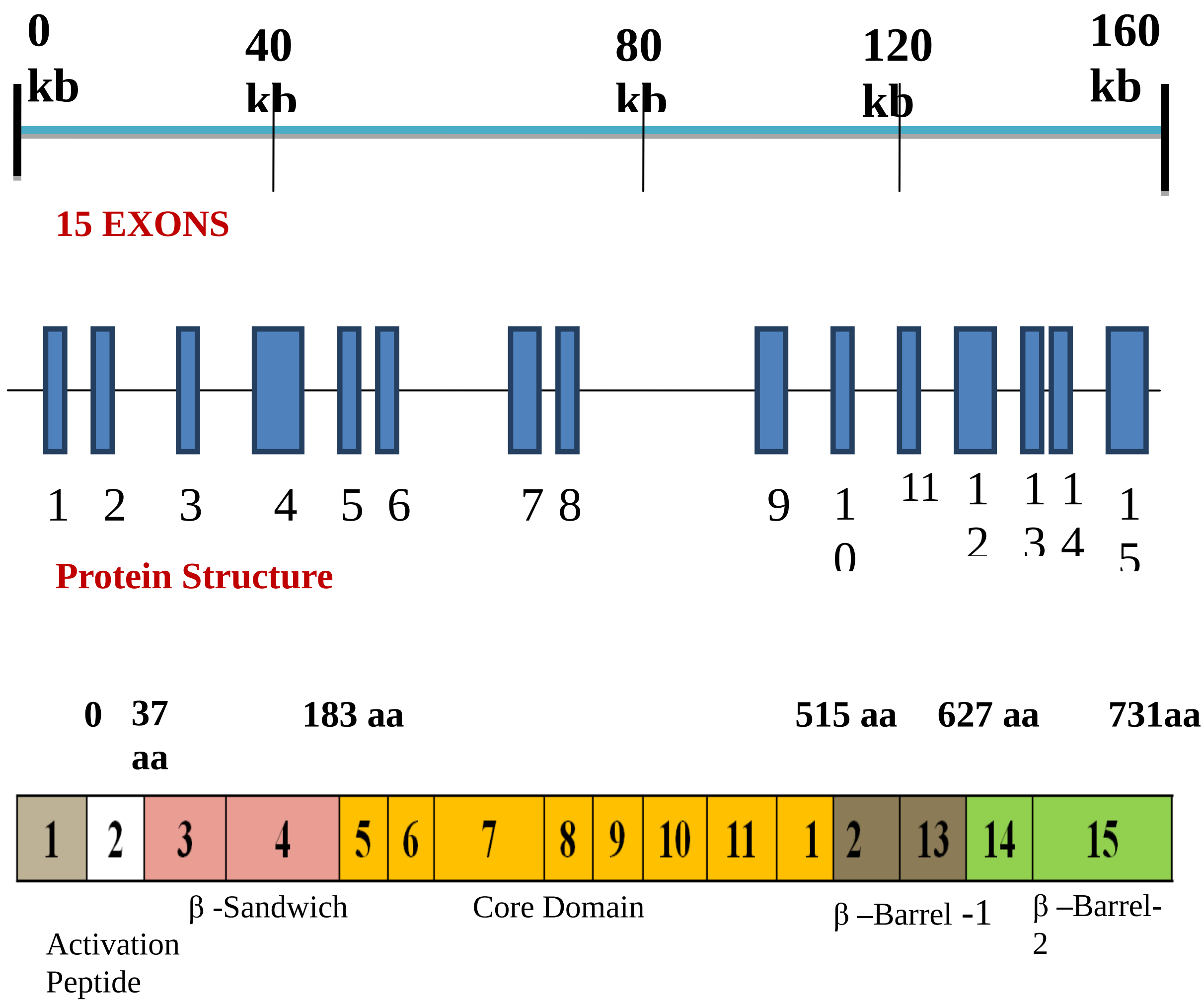

Figure 2.1 Factor XIII A: gene and protein structure

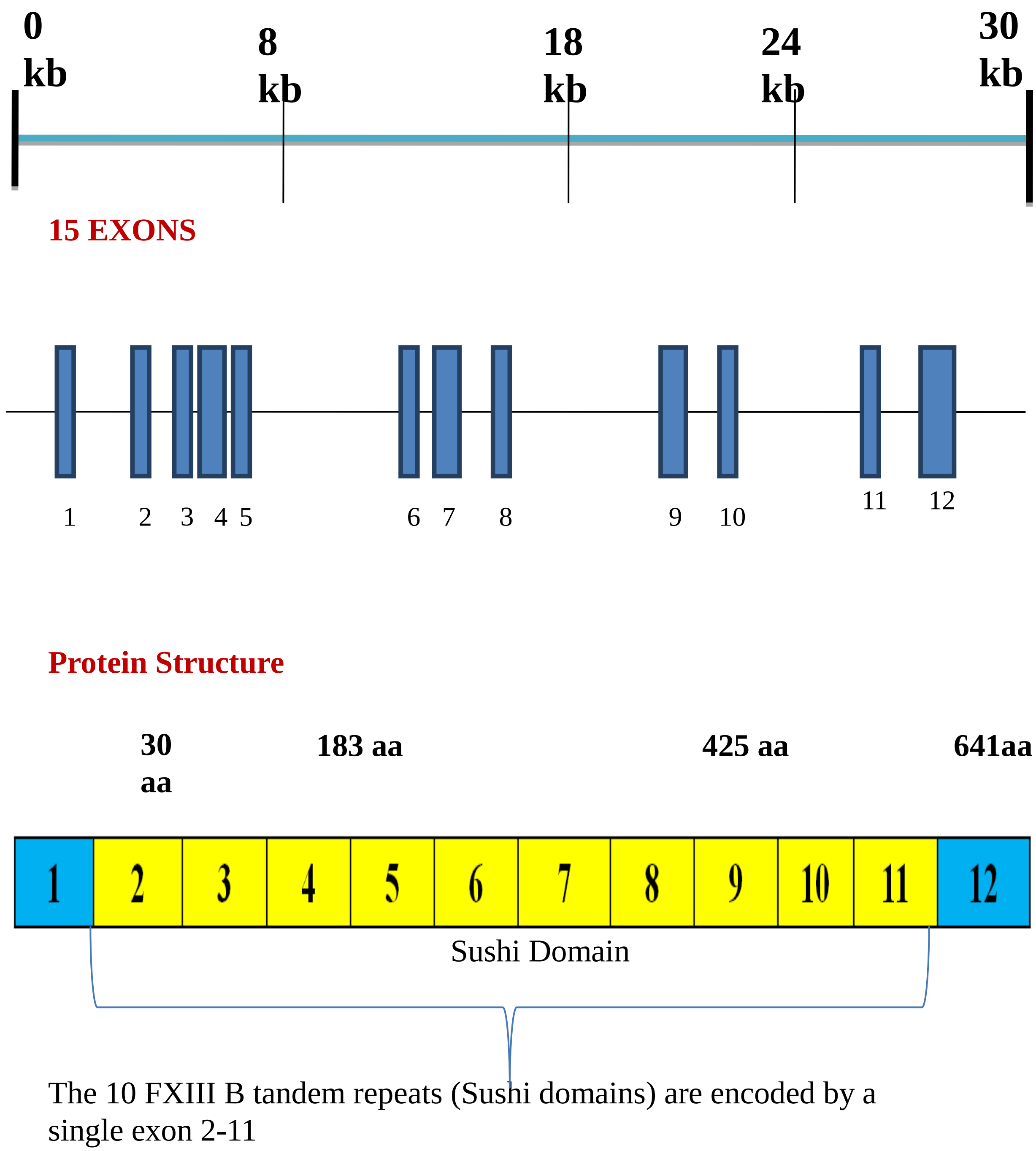

Figure 2.2 Factor XIII B: gene and protein structure

2.3 Factor XIII Function:

The main functions of FXIII are in three areas: Haemostasis, wound healing, and maintaining pregnancy (Muszbek et al., 1996). Other physiological functions of FXIII include immune defence, angiogenesis, and bone metabolism. In addition, it is involved in some pathological processes such as cardiovascular disease, inflammatory process, type 1 diabetes mellitus and neoplasm (Schroeder and Kohler, 2013).

2.3.1 Haemostasis:

The first step of FXIII activation occurs through proteolysis in which thrombin cleaves a scissile peptide bond between Arg37 and Gly38, and removes the N-terminal 37 amino acids (activation peptide), resulting in the formation of an active transglutaminase (FXIIIa) (Figure 2.3). In the presence of Ca^{2+} and fibrin, the B subunits then dissociate from the A subunits resulting in a conformational change, thus leaving the active site cysteine accessible for the substrate (McDonagh, 1994); FXIIIa then catalyses the cross-linking of fibrin or other target proteins which contain appropriate glutamine and lysine residues. FXIIIa covalently cross-links fibrin γ-dimers through incorporating reciprocal intermolecular ε-(γ-glutamyl) lysine bridges between the lysine at γ406 of one γ chain and a glutamine at γ398/399 of another fibrin molecule. In addition, $_{\alpha2}$plasmin inhibitor, a highly potent inactivator of plasmin, is also cross linked to the α-chain of fibrin and fibrinogen through FXIIIa, while still retaining its full plasmin inhibitory activity. The rapid cross linking of fibrin γ-chain homodimers and also linking $_{\alpha2}$ plasmin inhibitor with fibrin α-chain heterodimers are both followed by the slower progressive cross-linking of fibrin α-chains into high molecular weight polymers. Thus FXIIIa improves the mechanical strength, rigidity and elasticity of the clot and prevent it from being dissolved through fibrinolysis (Muszbek et al., 1996).

In addition to fibrin, FXIII also cross-links several other protein substrates in plasma and subendothelium, such as fibronectin, von Willebrand factor, vitronectin, collagen, coagulation factor V, thrombospondin and plasminogen activator inhibitor type 1 (PAI-1) (McDonagh, 1994; Hsieh and Nugent, 2008). Unlike plasma FXIII, the platelet FXIII is not activated through thrombin (proteolytic activation). Instead, the platelet FXIII undergoes a non-proteolytic activation following exposure to a target substance and rise intracellular calcium level (Polgar et al., 1990).

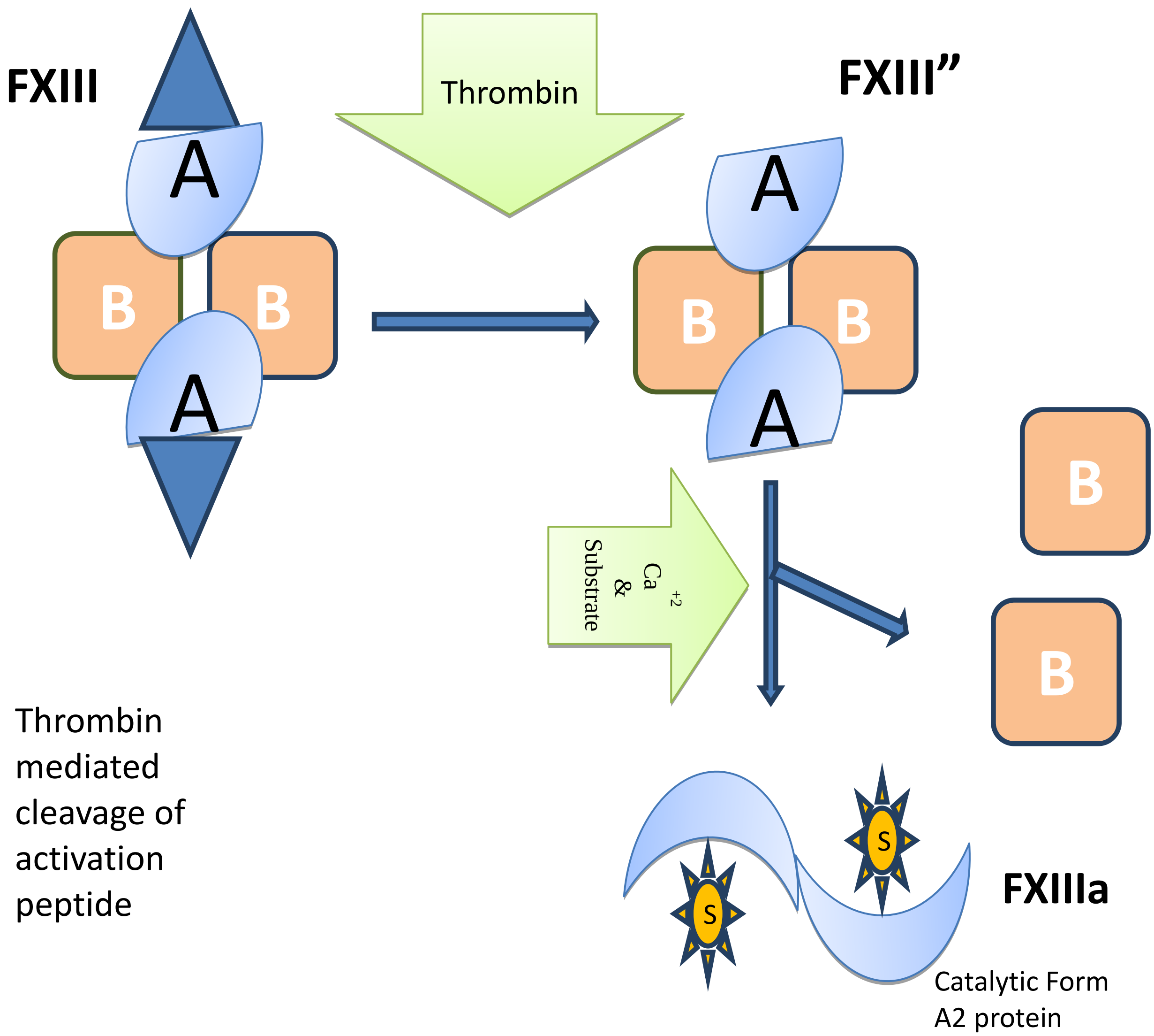

Figure 2.3 Plasma FXIII activation and fibrin cross-linking

2.3.2 Wound healing:

The role of FXIII in wound healing has been shown by impaired wound healing in a FXIII deficient patient (Vanscheidt et al., 1991) and in animal experiments (Inbal et al., 2005); favourable outcomes of FXIII application on wound healing and cell proliferation (Muszbek, 1999); antiapoptotic effect of FXIII (Dardik et al., 2003); effects on cell migration into the wound (Brown et al., 1993); and modulation of fibrin and connective tissue collagen biosynthesis (Paye et al., 1989). Animal experiments also suggest an important role of FXIII in improving wound healing following myocardial infarction and delay the development of heart failure (Nahrendorf et al., 2006). An interesting aspect of FXIII in the healing process was observed following myocardial infarction. It is suggested that FXIII assists in the healing process of ventricular wall following infarction through accelerating the resolution of neutrophil response, enhancing macrophage recruitment, increasing collagen content and promoting angiogenesis (Nahrendorf et al., 2006, 2008).

2.3.3 Immune defence and inflammation:

FXIII was found to interact with the complement immune system through the incorporation of C3 in the fibrin clot and prolongation of fibrinolysis. FXIII also contributes in preventing wound infection through sequestration of bacteria in the clot such as *Escherichia coli* and *Staphylococcus aureus* (Wang et al., 2010; Bagoly et al., 2012). This occurs through activated FXIII which cross-link bacterial surface proteins into fibrin fibers as observed from tissue biopsies in patients with necrotizing fasciitis (Loof et al., 2011). Another link between FXIII activity and the immune system comes from the observation of a lack of monocyte phagocytic activity in cases with FXIII deficiency even after converting to macrophages. The interaction of FXIII-A with platelet protein may enhance platelet binding to monocyte and enhance the localisation of immune response to the area of injury (Ichinose, 2012).

More recently, it was shown that human neutrophil elastase within neutrophil lymphocytes induce a limited cleavage of plasma or cellular FXIII and results in their activation, followed by much slower proteolytic inactivation (Bagoly et al., 2008).

2.3.4 Implantation and maintaining pregnancy:

FXIII-A has been found within macrophage cells present in the connective tissues of the endometrial and myometrial layers of uterine tissues obtained from normal women post hysterectomy (Adany and Muszbek, 1989). During embryogenesis, FXIII-A can be detected within chorionic mesenchyme of placenta where it appears inside the mononuclear round cells at the 5th week of gestation, or at the beginning of vasculogenesis. These small cells rapidly differentiate into large stellate cells and also increase in number from less than 10% of total cell number at the 5th week of gestation, to around 30% by the 7th week of gestation (Kappelmayer et al., 1994). FXIII containing cells cannot be detected in the circulation before the 9th week of gestation.

FXIII plays an important role in implantation that includes a complex interface between the uterus and blastocyst through the action of activated FXIII on cross linking fibrin, fibrinogen and fibronectin which is essential in placental attachment to uterine tissue (Wartiovaara et al., 1978). Implantation starts on the 7th day after ovulation. A week later, cytotrophoblasts and syncytiotrophoblasts invade the decidual stroma, forming a trophoblastic mass (Vićovac and Aplin, 1996). Two weeks after implantation, cytotrophoblasts undergo active proliferation, penetrate the trophoblastic mass and invade the decidual stroma resulting in the formation of extravillous cytotrophoblasts. From the 6^{th} to 8^{th} week of gestation, some extravillous cytotrophoblasts will develop into the cytotrophoblastic shell, and the rest of extravillous cytotrophoblasts infiltrate deeply into the decidual stroma, forming the interstitial cytotrophoblasts. The cytotrophoblastic shell makes the inner surface of the maternal–fetal binding interface, while the outer surface of the interface is formed by the Nitabuch's fibrinoid layer, all of which are well developed by the 8^{th} week of gestation (Asahina et al., 2000; Inbal and Muszbek, 2003). Kobayashi *et al* studied tissue samples obtained from three normal pregnant women for the presence of FXIII-A and FXIII-B; one sample was intrauterine implantation tissue obtained from a woman who underwent hysterectomy due to lyomyoma at 7 weeks of gestation, the second sample was tubal implantation tissue terminated during the 8^{th} week of gestation, and the last was a sample of placental tissue obtained from a woman with spontaneous miscarriage during the 14^{th} week of gestation. This study showed the presence of plasma-derived FXIII-A within the extracellular spaces of extravillous cytotrophoblasts, as well as the Nitabuch's layer adjacent to the cytotrophoblastic shell (Kobayashi et al., 1999).

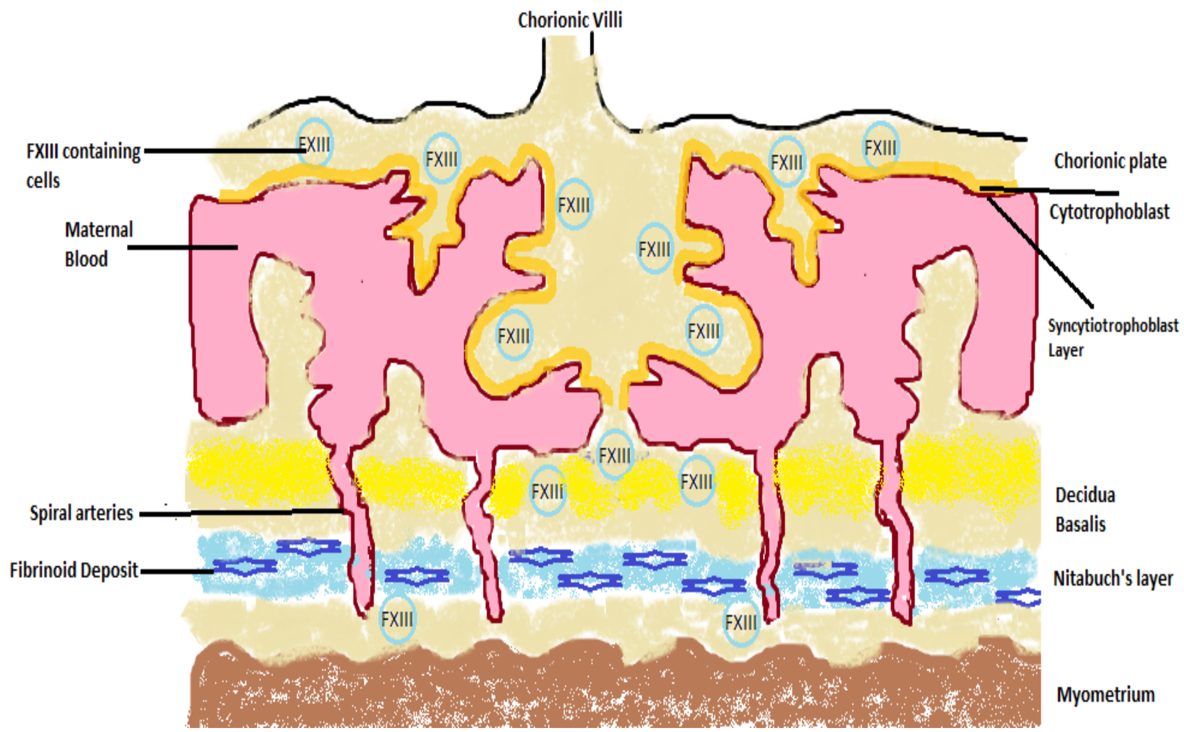

Figure 2.4 Role of FXIII in placental growth and development

2.4 FXIII deficiency

2.4.1 Congenital Factor XIII deficiency

2.4.1.1 Pathogenesis and classification

Congenital FXIII deficiency was originally classified (on the basis of antigen levels) into two categories; type I, characterised by deficiency of both FXIII-A and B subunits; type II, a deficiency of FXIII-A alone; and type III, a deficiency of FXIII-B alone (Girolami et al., 1978, 1991). However, genetic analysis concluded that type I deficiency is mainly due to a defect in *F13B gene* rather than a combined defect in *F13A* and *F13B genes* (Ichinose, 2001). As a result, the deficiency of FXIII-B leads to a loss of its function as a carrier for FXIII-A, and accelerates the clearance of FXIII-A from the circulation. This explains the reduction in FXIII-A level observed in type I defect (Muszbek et al., 2011a). Recently, the International Society on Thrombosis and Haemostasis, Scientific and Standardization Committee (ISTH SSC) classified congenital FXIII deficiency into two main types according to the genotype defect; FXIII-A deficiency, where the mutation involves *F13A gene*, and FXIII-B deficiency where the mutation involves *F13B gene*; The latter is rare and accounts for less than 5% of all cases of congenital FXIII deficiency. Both defects are characterised by an absence of the catalytic activity of FXIII in plasma. FXIII-A deficiency is further subdivided into type I, characterised by a quantitative defect resulting from decreased synthesis of the protein, and type II deficiency with a normal or near-normal concentration of functionally defective FXIII-A (Kohler et al., 2011; Muszbek et al., 2011a) (Table 2.1).

Table 2.1 Classification of FXIII deficiency according to the activity value and antigen concentration (Kohler et al., 2011).

Deficiency	Plasma FXIII activity	Plasma FXIII-A2B2 antigen	Plasma FXIII-A antigen	Plasma FXIII-B antigen	Platelet FXIII activity	Platelet FXIII-A antigen
Inherited						
FXIII-A deficiency						
Type I	↓↓↓	↓↓↓	↓↓↓	> 30%	↓↓↓	↓↓↓
Type II	↓↓↓	↓ -N	↓-N	> 30%	↓↓↓	↓-N
FXIII-B deficiency	↓↓	↓↓↓	↓↓	↓↓↓	N	N
Auto-Ab against FXIII						
Anti FXIII-A						
Neutralizing	↓↓↓	↓-N	↓-N	> 30%	N	N
Non-neutralizing	↓↓↓	↓↓↓	↓↓↓	> 30%	N	N
Anti FXIII-B	↓↓↓	↓↓↓	↓↓↓	↓↓↓	N	N
Other acquired deficiencies	↓	↓	↓	↓-N	N.A	N.A

↓↓↓, highly decreased activity/concentration usually below 30%; ↓↓, considerably decreased activity/concentration usually 5–10%; ↓, slightly decreased activity usually in the range of 20 –70%; N, normal; NA, non-applicable.

FXIII deficiency is inherited as an autosomal recessive trait with various clinical presentations, and those with severe disease are homozygotes or compound heterozygotes. It can affect people from all races and commonly there is a history of consanguinity within the families of affected patients. The first published genetic mutation leading to FXIII deficiency was reported by Webb et al. (Webb, 1989). At the time of writing this thesis, more than 100 causative mutations in FXIII genes have been reported. The number of reported gene mutations is 88 and 16 for the FXIII-A and FXIII-B genes respectively. Missense mutations are the commonest type of mutations, accounting for more than half of all the mutations in the FXIII A and B genes. There have been 46 missense, 23 deletions/insertions, 9 splice site, 10 nonsense mutations reported so far for the FXIII A gene (Biswas et al., 2011). More than 90% of the missense mutations occur at the catalytic core domain. A splice site mutation in intron 5 is the most frequent mutation reported in the FXIII-A gene in six unrelated families from UK, Netherlands, Czech Republic Macedonia, Serbia and Kosovo (Schroeder et al., 2006).

All details related to FXIII gene mutations are listed in different databases such as the Factor XIII Registry Database website (www.f13-database.de) and the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff website (www.hgmd.cf.ac.uk), and the ISTH website (www.isth.org). The mutations affecting the FXIII-A gene are mainly in the form of 62 missense, or nonsense, mutations (Table 2.2), in addition to other forms of mutations such as splice, insertion, and deletion (International Society on Thrombosis & Haemostasis, 2011). The most commonly known FXIII-A polymorphisms are in the form of Val34Leu, Tyr204Phe, Leu564Pro, Val650Ile, and Glu651Gln. In a case report, the FXIII-B gene defect was due to loss of the last five sushi domains in the B-subunit following a duplication in exon 7 (c.1155_1158dupACTT) (Ivaskevicius et al., 2010b). Other forms of FXIII-B mutations are listed in Table 2.3.

The common Val34Leu polymorphism is caused by defect in the FXIII gene characterised by a G-to-T transition (FXIII G103T) in exon 2 of the gene encoding for FXIII-A, resulting in a valine (V)-to-leucine (L) substitution at the 34th amino acid (Kobbervig and Williams, 2004). This form of gene mutation is not associated with changes in FXIII plasma concentration, but can alter FXIII activity. In carriers of FXIII G103T mutation, activation of FXIII-A by thrombin was found to occur two- to threefold more rapidly affecting clot stability. The thrombin-induced catalytic cleavage of FXIII-A changes the structure of the cross-linked fibrin, resulting in the formation of a fibrin clot made up of thinner fibers and weaker fibrin mesh. This mechanism has been suggested to be the underlying cause for increased risk of spontaneous miscarriage in women with Val34Leu polymorphism. (Dossenbach-Glaninger et al., 2003).

Table 2.2 FXIII-A missense /nonsense mutations (International Society on Thrombosis & Haemostasis, 2011).

Exon	Amino acid change	Domain		Reference
2	His64Tyr	b-sandwich		Ivaskevicius et al. 2007
3	Asn60Lys Arg77Cys Arg78Cys Arg77His Glu102Lys Tyr69stop	b-sandwich b-sandwich b-sandwich b-sandwich b-sandwich b-sandwich		Anwar R et al. 1995 Duan et al. 2002 Halverstadt et al. 2006 Peyvandi et al. 2004 Anwar 2002 Jayandharan et al 2006
4	Met159Arg Arg171Stop Arg174Stop Pro186Leu	b-sandwich b-sandwich b-sandwich Core		Schroeder et al. 2006 Standen and Bowen 1993 Zheng et al. 2009 Castaman et al. 2008
5	Gly210Arg Gly215Arg Y204Stop	Core Core Core		Vesokovsky et al. 2004 Schroeder et al. 2006 Souri et al, 2012
6	Leu235Arg Met242Thr Arg252Ile Arg260Cys Arg260Leu Arg260His Gly262Glu Ser263Phe	Core Core Core Core Core Core Core Core		Birben et al. 2003 Mikkola et al. 1994 Mikkola et al. 1996 Ichinose 1998 Vysokovsky et al, 2004 Kangsadalamai 1999 Onland et al. 2005 Jayandharan et al. 2006
7	Tyr283Cys Ser295Arg Val316Phe Ala318Val Lys257Glu Pro289Arg	Core Core Core Core Core Core		Souri et al. 2001 Anwar R et al. 2000 Onland et al. 2005 Vysokovsky et al, 2004 Ivaskevicius et al. 2007 Ivaskevicius et al. 2010
8	Arg326Gln Arg326Stop Leu354Pro	Core Core Core		Mikkola et al. 1996 Anwar 2005 Anwar et al. 2001
9	Trp375Cys Ala378pro Arg382Ser Ala394Val Thr398Asn Gln400Stop Gly390Stop	Core Core Core Core Core Core Core		Schroeder et al. 2006 Ivaskevicius et al. 2007 Peyvandi et al. 2003 Izumi 1998 Vysokovsky et al, 2004 Kangsadalampai et al 1996 Ivaskevicius et al. 2010
10	Arg408Gln Ser413Leu Ser413Trp Val414Phe Gly420Ser Tyr441Stop	Core Core Core Core Core Core		Anwar R et al. 1995 Niya et al. 1999 Duan et al. 2003 Aslam 1997 Kangsadalampai et al 1996 Anwar. 1995
11	Gly501Arg Tyr441stop Thr449Ile	Core Core Core		Board 1993 Anwar R et al. 1995 Maak et al, 2010
12	Leu498Pro Gly501Arg Gly562Arg Asn541Lys Arg430Gln	Core Core Barrel 1 Barrel 1 Barrel 1		Mikkola et al. 1996 Board 1993 Takahashi et al., 1998 Birben et al. 2002 Ivaskevicius et al. 2010
13	Leu604Pro Gly592Ser, Arg611His	Barrel 1 Barrel 1		Ivaskevicius et al. 2007 Ivaskevicius et al. 2010
14	Leu660Pro Arg661Stop Leu667Pro Trp664stop, Asp668Gly	Barrel 2 Barrel 2 Barrel 2 Barrel 2		Inbal et al. 1997 Mikkola et al. 1994 Aslam 1995 Ivaskevicius et al. 2010
15	Trp691Stop His716Arg Arg703Trp Ser708Asn S708R	Barrel 2 Barrel 2 Barrel 2 Barrel 2 Barrel 2		Anwar 2005 Schroeder et al. 2006 Wu et al. 2006 Castaman et al, 2008 Souri et al, 2012

Table 2.3 FXIII-B mutations (International Society on Thrombosis & Haemostasis, 2011)

Location	Amino acid change	Domain	Reference
Intron 1	IVS1-2 del A*		Koseki et al, 2001
Intron 1 Exon 8	IVS1-2 del A* Cys430Phe	 Sushi 7	Saito et al, 1990
Intron 1 Exon 9	IVS1-2 del A* nt 1498 del G	 Sushi 8	Koseki et al, 2001
Exon 2	Cys5Arg	Sushi 1	Ivaskevicius et al, 2010
Intron 2	IVS2-1 G>C		Ivaskevicius et al, 2010
Exon 3	nt 299 ins AAC*	Sushi 2	Izumi et al, 1996 Souri et al, 1998
Exon 3	Ile81Asn*	Sushi 2	Saito et al, 1990
Exon 3	Leu116Phe	Sushi 2	Ivaskevicius et al, 2010
Intron 3	IVS3 -1 G>C		Ivaskevicius et al, 2010
Exon 4	nt 471 del ATT	Sushi 3	Ivaskevicius et al, 2010
Exon 5	Val217Ile	Sushi 4	Ivaskevicius et al, 2010
Exon 7	Cys316Phe*	Sushi 6	Ivaskevicius et al, 2010
Exon 7	nt 1158 ins ACTT	Sushi 6	Ivaskevicius et al, 2010
Exon 8	Val401Glu	Sushi 7	Ivaskevicius et al, 2010
Exon 8	Pro428Ser	Sushi 7	Ivaskevicius et al, 2010
Exon 12	Nt1959 ins T	Sushi 12	Ivaskevicius et al, 2010

2.4.1.2 Prevalence and Clinical manifestations

Congenital FXIII deficiency, where there is < 1% of the normal level of FXIII activity, is a rare bleeding disorder with an estimated incidence of one in 1-2 millions (Peyvandi et al., 2009). Most cases of congenital FXIIII deficiency are characterised by a severe bleeding tendency, as well as poor wound healing and spontaneous miscarriage among affected women (Kobayashi et al., 1990; Asahina et al., 1998, 2000, 2007; Burrows et al., 2000; Dardik et al., 2006).

The earliest manifestation of this condition is in form of umbilical bleeding presenting few days after delivery, making it a characteristic sign and the most common symptom reported in about 80% of the cases (Anwar and Miloszewski, 1999; Anwar et al., 2002). Extensive bleeding following minor trauma has been found to be significantly associated with heterozygous deficiency of FXIII. Bleeding into the skin and subcutaneous tissues can lead to extensive bruises (Mahmoodi et al., 2011). Intracranial haemorrhage (ICH) occurred in up to 30% of reported cases. This is more frequent than in any other inherited clotting disorder, such as haemophilia A or B, and is considered the leading cause of mortality or morbidity among patients with congenital FXIII deficiency (Duckert, 1972; Karimi, 2009). In addition, mouth and gum bleeding following tooth extraction can be a recurrent and serious problem. Bleeding into muscles and the periarticular area is also common and can be severe requiring hospital admission. Characteristically, intramuscular bleeding can occur following heavy exercise, even without any history of direct trauma (Anwar and Miloszewski, 1999). The frequency of bleeding in patients with congenital FXIII deficiency are summarised in Table 2.3. While menorrhagia is considered a common and major symptom in many bleeding disorders like von Willebrand disease and Factor XI deficiency, its prevalence and impact among women with FXIII deficiency is still not clear (Peyvandi et al., 2011b). In a case series by Lak *et al* from Iran, a history of menorrhagia was reported in 7/20 (35%) women with congenital FXIII deficiency (Lak et al., 2003). In addition, a review of published case reports by Burrow *et al* in 2000 showed Menorrhagia as a clinical symptom in 7/11 (64%) women with FXIII deficiency (Burrows et al., 2000).

A recent study on the European Network of Rare Bleeding Disorder proposed a grading system of bleeding among patients with coagulation disorder (Peyvandi et al., 2012):

1) Asymptomatic: No documented bleeding episode
2) Grade I bleeding: Post-traumatic or post drug ingestion
3) Grade II bleeding: Spontaneous minor bleeding (bruising, ecchymosis, oral cavity bleeding, epistaxis and menorrhagia)
4) Grade III bleeding: Spontaneous major bleeding (Intramuscular haematoma, haemarthrosis, CNS, Gastrointestinal and umbilical cord bleeding)

Based on the same study above, FXIII activity that was necessary for patients to remain asymptomatic was 31 IU/dL. For those with Grade I bleeding the mean FXIII activity was 16.85 IU/dL, for Grade II was 2.6 IU/dL, and Grade III was 0.IU/dL (Peyvandi et al., 2012).

Table 2.4 Bleeding sites in patients with congenital FXIII deficiency (Anwar and Miloszewski, 1999).

Bleeding site	Percentage
Umbilical bleeding	80
Superficial bruising	60
Subcutaneous hematoma	55
Menorrhagia	35 – 64
Mouth and gums	30
Intracranial haemorrhage	25-30
Muscles	27
Lacerations	24
Joints	24
After surgery	17
Peritoneal	14
Epistaxis	10
Genital	9
Renal	8
Peripheral nerves	6
Eye, Gastrointestinal, spleen	3
Ears	2
Pleural	1

2.4.2 Acquired FXIII deficiency

Acquired FXIII deficiency develops through two main mechanisms: either due to the formation of auto-antibodies against a FXIII subunit or due to a reduction in the synthesis of a FXIII subunit as a result of impaired bone marrow function or liver disease, over consumption, or dilution coagulopathy; the latter result in a moderate FXIII deficiency. Up to 2011, more than 50 cases of FXIII deficiencies due to an anti-FXIII-A autoantibody were reported in the literature (Muszbek et al., 2011a). In most cases, the formation of anti-FXIII autoantibody is idiopathic and commonly seen in elderly patients. In about 30% of the cases, the autoantibody develops in patients with an autoimmune disorder, especially in cases with systemic lupus erythematosus. Some medications have also been associated with acquired FXIII deficiency including isoniazid (Otis et al., 1974), penicillin (Lopaciuk et al., 1978), phenytoin (McDevitt et al., 1972), and practolol (Milner et al., 1977). Formation of antibodies in cases of congenital FXIII is very rare and has been observed in few cases receiving frequent transfusion (Henrikson et al., 1983).

Auto-antibodies against FXIII-A are of IgG type, either neutralising or non-neutralising. Neutralising FXIII-A auto-antibodies act through interfering with FXIII activation, while non-neutralising autoantibody work through developing an immune complex with the FXIII subunit, resulting in a rapid clearance of the complex through the reticulo-endothelial system. It was noted that most of the early reported cases of acquired FXIII deficiency were claimed to be due to neutralising auto-antibodies. However, this might be a result of difficulties in identifying non-neutralising auto-antibodies (Muszbek et al., 2011a). In a systematic review of patients in Japan reported with acquired FXIII deficiency, 21 patients were identified (13 male and eight females). In ten cases, FXIII activities were below 5

IU/dL. Intramuscular and subcutaneous bleeding was the most common symptom, seen in half of the cases (Ichinose and Souri, 2011).

FXIII concentrate infusion has been used in patients with acquired factor XIII deficiency during acute bleeding with or without blood transfusion, while fresh frozen plasma and cryoprecipitate are less successful (Nakamura et al., 1988). Plasmapheresis might be used to improve the symptoms by reducing the auto-antibody level. Some immunosuppressants, such as cyclophosphamide, have been used for managing cases of acquired FXIII deficiency by reducing the antibody concentration and improving FXIII function. The same therapy has been used for other hematologic disorders with an autoimmune mechanism such as autoimmune haemolytic anaemia, chronic immune thrombocytopenia, and acquired factor VIII inhibitors. Rituximab, has been rarely used in the treatment protocol to inhibit B-cell proliferation and cell medicated cytotoxicity (Ichinose and Souri, 2011).

2.4.3 Diagnosis:

The diagnosis of congenital FXIII deficiency is based on the clinical history of bleeding diathesis, especially delayed umbilical cord bleeding, in the presence of normal laboratory coagulation screening tests, such as prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen level, platelet count and bleeding time. The following algorithm has been recommended to diagnose and classify FXIII deficiency (Karimi, 2009; Kohler et al., 2011):

A- A screening test for the detection of FXIII deficiency using a quantitative FXIII activity assay.

B- If FXIII activity with the above test is found to be reduced, further tests are performed to detect the subtype of FXIII deficiency. These include:

  a. Measurement of plasma level of FXIII-A2B2 antigen, If FXIII-A2B2 antigen concentration is decreased,

  b. Measurement of FXIII activity and FXIII-A antigen in the plasma and in platelet lysate and measurement of FXIII-B concentration in plasma.

C- FXIII-subunits auto-antibody assessment through:

  a. Detect neutralising antibodies against FXIII-A

  b. Binding assays to detect non-neutralizing antibodies against FXIII-A and FXIIIB.

D- Evaluation of the presence of FXIII inhibitors by analysing cross-linked fibrin using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

E- Study of the molecular genetic defect.

2.4.3.1 Non Quantitative assay – Urea clot solubility assay

The urea clot solubility was the first test used in the investigation of FXIII deficiency and is still used to diagnose cases who have FXIII levels of <1%. A survey conducted in 2002 by the UK National External Quality Assessment Scheme for Blood Coagulation (UK NEQAS) revealed that 125 out of 150 (83%) of UK laboratory centres continued to use a clot-solubility screening test(Jennings et al., 2003). The test involves incubating the plasma with thrombin with or without calcium ions until a clot is formed. The test is considered positive when the clots dissolve following the addition of 5 M urea, 2% acetic acid or 1% monochloracetic acid. The test sensitivity can vary from <0.5% to 5% FXIII activity based on the fibrinogen level, the solubilising agent concentration, and the clotting reagent used (thrombin, Ca^{2+} or a combination of the two) (Jakobsen and Godal, 1974; Francis, 1980; Jennings et al., 2003). A study on the sensitivity of clot solubility test recommended the use of a combined thrombin/acetic acid in the clot solubility test as the thrombin/acetic acid is sensitive to a minimum FXIII activity of 10 IU/dL compare to the calcium/urea method which is sensitive in detecting FXIII activity between 1 and 5 IU/dL, with an intermediate sensitivity for the calcium/acetic acid and thrombin/urea methods (Jennings et al., 2003). The test has many disadvantages as it is poorly standardised and relatively insensitive, detecting only severe deficiency (i.e. <5 IU/dL FXIII activity). The use of this method alone in screening for FXIII deficiency contributes to the underestimation of the prevalence of this condition because this test would be normal in mild FXIII deficiency with FXIII levels above 10-30 IU/dL (Mackie et al., 2013).

2.4.3.2 Quantitative FXIII activity assays:

Quantitative FXIII activity assays are based on activating FXIII in plasma using thrombin and Ca^{2+} , followed by the measurement of the transglutaminase activity of FXIIIa using two assay principles: the measurement of ammonia released during the transglutaminase reaction (chromogenic or ammonia release assays), and the measurement of labelled amine incorporated into a protein substrate (amine incorporation assays) (Karimi, 2009).

2.4.3.2.1 Ammonia release assay:

In the ammonia release assay, the clotting of plasma is inhibited by a fibrin polymerisation inhibitory peptide. FXIIIa then cross links a small molecular weight substrate amine to a glutamine-containing oligopeptide, and ammonia released during this transglutaminase reaction is measured with an NAD(P)H-dependent glutamate dehydrogenase reaction using photometric method and at a wavelength of 340nm (Muszbek et al., 1985). The Berichrom FXIII (Siemen's Healthcare, Marburg, Germany) and REA-chrom FXIII (Reanal, Budapest, Hungary) both measure FXIII activity using the ammonia release assay principle. The difference between the two assays is in the cofactor used, which is NADH in the Berichrom FXIII assay, while REA-chrom FXIII uses NADPH as a cofactor for the indicator reaction (Ajzner and Muszbek, 2004). The advantage of ammonia release assays is that they are true kinetic assays and can be easily automated and performed in one step using single reagent, in addition to being rapid, taking less than 10 minutes to produce results. Their disadvantage is the relatively low sensitivity, as they fail to give measurable results when FXIII is less than 5% for the Berichrom FXIII, or less than 3% for the REA-chrom FXIII assay (Karimi, 2009). The specificity of FXIII activity assays can be affected by the presence of some side-reactions that result in the release of ammonia or the consumption of NADPH independent of FXIIIa activity, with 2-15 % overestimation of FXIII activity. For this reason, plasma blank solution are recommended to be measured and their value is subtracted from the test plasma readings to obtain correct FXIII activity results and prevent systematic overestimation (Ajzner and Muszbek, 2004). A plasma blank is provided with the REA-chrom FXIII kit or prepared by the user of Berichrom FXIII.

2.4.3.2.2 Amine incorporation assays

The amine incorporation assays are based on the formation of a covalent bond between a labelled amine and a glutamine residue in a protein substrate by FXIIIa. Following the termination of the reaction, the unbound amines are removed, leaving the protein-linked fraction to be measured quantitatively. The amine incorporation method has a high sensitivity when compared with the ammonia released assays. However, they are more time consuming, difficult to standardize, and the separation step makes it unlikely to perform a true kinetic assay (Karimi, 2009; Kohler et al., 2011). The Pefakit FXIII incorporation assay (Pentapharm, Basle, Switzerland) is a commercially available kit that measures the initial stage of FXIII transglutaminase activity based on the incorporation of 5-biotinamidopentylamine into surface-adsorbed fibrinogen (Kohler et al., 1998; Wilmer et al., 2001).

Using a low thrombin concentration of 1U/mL, the Pefakit FXIII assay produces a partial activation of plasma FXIII and can detect differences in the rate of FXIII activation in cases with FXIII-A Val34 to Leu polymorphism. When using the Pefakit FXIII assay, each genotype would have a different activation rate and subsequently different FXIII activity readings. The Leu34 variant in homozygote individuals is activated significantly faster (151- 483%) than the heterozygous (97 -251%) and Val34 wild-type individuals (46 - 200%). A disadvantage of this assay is that it correlates poorly with the antigenic assay (Karimi, 2009; Kohler et al., 2011).

The FXIII Standardization Working Party of the ISTH Scientific and Standardization Committee (SSC) considered the establishment of a reference material with a standard potency for both FXIII activity and antigen and thus reduces the variations between different laboratories and help in unifying their results. For this purpose, the SSC developed the World Health Organisation (WHO) reference plasma with an assigned FXIII activity and FXIII-A2B2 antigen values in International Units to be the 1st International Standard for Factor XIII, Plasma (IS). The WHO reference plasma is available from the National Institute of Biological Standards (NIBS) (Potters bar, UK) to be used for the calibration of reference plasmas by companies manufacturing FXIII assay kits (Raut et al., 2007).

An abnormal level of FXIII of 1% or less using a clot solubility test has been correlated with delayed umbilical bleeding and has a high risk of ICH in the first decade of life (Hsieh and Nugent, 2008). However, it is still difficult to correlate FXIII activity with the severity of other bleeding symptoms because the limitations of methods to accurately detect < 5% FXIII activity. Therefore, more sensitive assays of FXIII activity are required in order to determine which patients are at highest risk of bleeding based on their FXIII level (Hsieh and Nugent, 2008; Muszbek et al., 2011a).

2.4.3.2.3 Fluorometric FXIII assay*:*

This is based on the isopeptidase activity of human plasma FXIII and useful in detecting plasma FXIII activity < 5 IU/dL. In this method, the increase in emission resulting from an N-terminal attached fluorophore is observed continuously. An internally attached dark quencher is hydrolysed from a substrate peptide via the isopeptidase activity of FXIIIa. This method allows measurement of FXIIIa activity in a kinetic mode over a broad range of time and concentrations without reduction of the substrate. In comparison to the photometric assay, the fluorometric assay was found to be suitable for determining plasma FXIII levels in healthy individuals (Oertel et al., 2007).

2.4.3.3 FXIII Ag assays:

FXIII Antigen assays are needed for the purpose of classifying FXIII deficiencies. One method involve using ELISA test, such as R-ELISA FXIII (Reanal-ker, Budapest, Hungary). This example consists of a one-step ELISA test for detecting complexed FXIII in diluted plasma using horseradish peroxidase labelled monoclonal antibody detector against FXIII-A, and biotinylated monoclonal capture antibody to detect FXIII-B (Katona et al., 2000). The test is considered highly sensitive, with an ability to determine FXIII levels from 0.1% and above, making it a test of choice in determining FXIII traces among patients with homozygous FXIII deficiency. In addition, R-ELISA FXIII is useful for monitoring plasma FXIII concentration following replacement therapy with FXIII concentrates, with a reference interval of 14.0 to 28.0 mg/L, or 69 to 133% of normal average plasma FXIII concentration, based on the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS).

Another immunoassay method for determining FXIII-A and B antigen level relies on latex-enhanced immune precipitation (Instrumentation Laboratory, Milano, Italy). The main aim of immunoassay methods is to obtain identical results with noncomplexed FXIII-A and with FXIII-A in complex with FXIII-B and that binding of FXIII to fibrinogen had no influence on the assay results (Katona et al., 2000; Katona E et al., 2001).

2.4.4 Treatment of Factor XIII deficiency

2.4.4.1.1 Treatment products

The early cases of FXIII deficiency were treated using whole blood, cryoprecipitate and fresh frozen plasma (FFP) as a source of FXIII. Since the half life of endogenous FXIII is long, ranging from 5 to 11 days, prophylactic therapy has been given in form of FFP doses of 10 mL kg^{-1} every 4 to 6 weeks and cryoprecipitate in doses of one bag per 10–20 kg every 3 to 4 weeks . However, these therapies are associated with a risk of blood born diseases such as hepatitis, HIV, West Nile virus and other viruses and it is recommended that treatment should be, when possible, with FXIII concentrates (Gootenberg, 1998; Hsieh and Nugent, 2008).

Taking advantage of the high concentration of FXIII in placental tissue (10 folds greater than the plasma level), the first placenta-derived FXIII concentrate was developed in 1970 with a similar half life to plasma FXIII, but it only contained FXIII-A2 homodimer without FXIII-B. The recommended dose was10 IU/kg body weight per month for type II congenital FXIII deficiency, and higher doses (about 15 IU/kg body weight) were needed for patients with type I FXIII deficiency since the infused FXIII transamidase activity was lower in this group of patients (Rodeghiero et al., 1991). However, its use was decreased in 1992 following the availability of plasma FXIII concentrate.

2.4.4.1.1.1 Plasma Factor XIII concentrates

Plasma FXIII concentrate (Fibrogammin P; Dade Behring, Marburg, Germany) combine both FXIII-A and FXIII-B subunits. This concentrate was developed in the 1980s. It is a highly purified, heat-treated, lyophilised preparation and obtained from a human FFP after being tested and found negative for hepatitis, HIV, and high levels of alanine amino-transaminase. Fibrogammin P is usually provided as a freeze-dried powder stored in vials containing 1250 units that is equal to the amount of FXIII obtained from 1250 ml of plasma (Gootenberg, 1998).

The first multicentre prospective study on Fibrogammin P was conducted in France and involved 16 patients with male to female ratio of 3:1 and aged from 2 weeks to 46 years on regular infusions over variable periods ranging from19–108 weeks. The study reported a good tolerance toward the therapy with no detectable anti FXIII inhibitors. There was no reported major bleeding episode. Two patients reported some side effects in form of headache during menstrual cycle and a transient urticarial reaction (Dreyfus et al., 2003).

A more recent ongoing study on Fibrogammin P in the United States included 61 patients, male to female ratio of 3:1 and a mean age of 12.7 years (ranging from 1 to 74 years). Patients who received monthly prophylaxis of Fibrogammin P for a period ranging from 9.5 to 121 months had a favourable response with no development of inhibitors or seroconversion. The patients experienced no bleeding episode while being under prophylaxis, including a woman who had an uneventful pregnancy while receiving Fibrogammin P every three week (Nugent, 2006; Lusher et al., 2010). Furthermore, Castaman et al reported six patients with FXIII deficiency with no adverse effects following the administration of more than 1,200 prophylactic infusions of FXIII concentrate in a dose 10 IU/kg per month during a maximum period of 20 years (Castaman, 2008). Therefore, Fibrogammin P is considered to be a convenient source of FXIII for patients with FXIII deficiency with an ability to produce normal clotting pattern according to thromboelastography (Nugent, 2006; Lusher et al., 2010; Dreyfus et al., 2011). Currently, the product is approved for use in the treatment of patients with FXIII deficiency in several countries including UK, Japan, and Germany and was only approved by the Food and Drug Agency (FDA) to be used in the U.S in 2011(Behring CSL, 2011). It is recommended by the manufacturer to be administered every 4 to 6 weeks at a dose of 10U/kg for prophylaxis against bleeding, and up to 35 U/kg prior to surgical procedures (Behring CSL, 2011). Fibrogammin P dosage can vary widely according to patient response and pharmacokinetics. This variation can be attributed to the heterogeneity of FXIII deficient patients, the presence or absence of intracellular FXIII subunits, and the existence of polymorphisms that can alter FXIII activity (Hsieh and Nugent, 2008).

2.4.4.1.1.2 Recombinant Factor XIII concentrates

The recombinant FXIII-A2 (rFXIII-A2) homodimer is developed in *Saccharomyces cerevesiae* and contain no human or mammalian products in the culture or media. Following administration of rFXIII-A2, the rFXIII-A2 homodimers rapidly combine with free plasma FXIII-B subunit to form stable FXIII-A2B2 heterotetramer with a terminal half life of 9 to 13 days. The first recombinant FXIII concentrate was developed in the early 1990s (ZymoGenetics Incorporated, Seattle, Washington) but was not tested in any clinical trial. In 2005, a study of the safety and pharmacokinetic of rFXIII-A2 administered to 50 healthy volunteers using a dose range from 25 to 50U/kg found an increase in the rFXIII-A2 plasma activity to 64-88% above baseline (Reynolds et al., 2005; Visich et al., 2005).

This was followed by a phase one clinical study of the drug pharmacokinetic and safety profile using a single dose rFXIII-A2 in 10 adults affected with congenital FXIII-A deficiency. The patients in the study had an increase in their FXIII plasma activity of 57 to 59 IU/dL, 105 to 129 IU/dL, and 160 to 181 IU/dL following the administration of 20 U/kg, 50 U/kg, and 75 U/kg rFXIII respectively, with a median dose response of 2.4% increase in FXIII activity for every unit of rFXIII given per kilogram body weight. Furthermore, the stable FXIII-A2 B2 formed as a result of rFXIII administration in this study had a half life of 8.5 days similar to that of endogenous plasma FXIII, with no adverse effects or antibody formation against rFXIII. However, rFXIII concentrate administered to patient with FXIII-B subunit deficiency had a shortened terminal half-life of 8.9 hours, and an increased clearance compared with the patients with FXIII-A2 deficiency (Lovejoy et al., 2006).

2.4.4.1.2 Treatment of acute bleeding

For the management of acute bleeding in patients with FXIII deficiency, treatment with FXIII concentrate is recommended in a dose of 10–20 units per kilogram body weight. Regular monitoring of plasma levels is important to maintain plasma FXIII levels above 10-20 IU/dL until the bleeding is controlled.

2.4.4.1.3 Regular Prophylaxis

Prophylactic factor replacement is mandatory in patients with FXIII deficiency and previous history of ICH. Prophylactic therapy is also recommended to patients with severe FXIII deficiency (FXIII level <1 IU/dL) and considered in those patients with FXIII level <4 IU/dl (Anwar et al., 2002; Bolton Maggs et al., 2004). Castaman suggested that FXIII level of 5 IU/dL is sufficient to prevent spontaneous bleeding (Castaman, 2008). It was also recommended that maintaining plasma FXIII level of 3-10 IU/dL is required to prevent life threatening bleeding such as spontaneous ICH (Anwar et al., 2002). The United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) in its latest (2004) guidelines recommend FXIII concentrate in a dose of 10 units /kg body weight at 4 weekly intervals to provide adequate prophylaxis for patients with severe FXIII deficiency(Bolton Maggs et al., 2004).

2.4.4.1.4 Prophylaxis during surgery

Surgery and other invasive procedures can be managed by providing sufficient amount of replacement therapy prior and during the procedure using FXIII concentrate or, if concentrate is not available, large amounts of FFP. In a major surgery, a daily dose of 20–30 U per kilogram body weight is recommended shortly before the operation, while for a minor surgery, a daily dose of 10-20 U per kilogram body weight is needed. Following the surgery, the plasma FXIII level is monitored, aiming to achieve FXIII level above 5 IU/dL for 5 days after the surgery or until the wound has healed completely (Bolton Maggs et al., 2004). However, there are no prospective or controlled trials to validate the recommended FXIII level needed in these settings.

2.4.4.1.5 Prophylaxis during pregnancy and labour

Pregnant women with FXIII deficiency can develop deicidual bleeding as early as the 5th or 6th week of gestation resulting in miscarriage unless prevented using replacement therapy. The aim of the perinatal management is to keep FXIII-A levels higher than 10 IU/dL, or at least 2-3 IU/dL to avoid miscarriage (Asahina et al., 2007). The UKHCDO recommended regular prophylaxis in pregnancy with monitoring plasma FXIII level, aiming to keep the trough level >3 IU/dL. For this purpose, FXIII concentrate is the treatment of choice rather than FFP or cryoprecipitate (Bolton Maggs et al., 2004).

FXIII concentrate is recommended to be administered in a dose of 250 U weekly during the first 22 weeks of gestation and to be increased to 500 IU weekly from 23 weeks of pregnancy onward. The aim of the treatment is to maintain FXIII-A levels above 10-20 IU/dL throughout pregnancy (Asahina et al., 2007; Peyvandi et al., 2011a). Meanwhile, during labour, a dose of 1000 U of FXIII concentrate has been recommended to achieve a level greater than 20-30 IU/dL needed to prevent extensive obstetrical bleeding, including primary postpartum haemorrhage (PPH) and wound bleeding. Administration of FXIII concentrate during labour would also reduce the chance of developing secondary PPH considering the long half life of plasma FXIII (Ichinose et al., 2005; Hsieh and Nugent, 2008).

2.4.5 Obstetric and gynaecological manifestation of FXIII deficiency

2.4.5.1 Menstrual cycle

The histology of the uterus consists of endometrium, myometrium and perimetrium. The endometrium forms the inner layer of uterus and is made of a specialised mucosal layer comprising a basal and superficial layer of glandular epithelium as well as cellular stroma. It is constantly undergoing cyclical changes in form of growth, differentiation and finally cyclical shedding leading to menstruation, all in response to changes in the sex hormone throughout a woman's reproductive life (Fusi et al., 2006).

The first day of the menstrual cycle begins with the initiation of menstrual flow. The menstrual phase, lasting usually between 4 to 6 days, is characterised by the shedding of the thickened endometrium to form menstrual bleeding. Days 7 through 14 represent the follicular or proliferative phase, which conclude in ovulation. The luteal, or secretory phase lies between days 15 and 28 (Farage et al., 2009). The follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both secreted from the pituitary, play an important role in the morphological changes in the ovaries and uterus during menstruation. Following menstruation, LH together with follicle-stimulating hormone initiates production of several primary follicles, which produce estradiol as well as other estrogens, leading to an increase of endometrial thickness. The estradiol level starts to steeply increase about a week before ovulation, reaching its maximum one day before the LH peak (one day before ovulation), followed by a rapid decline. After ovulation, the corpus luteum is formed in the ovaries which produce progesterone in increasing amounts, rising up to 10 fold during the week following ovulation and making it the dominant hormone during the early and mid-luteal phase. As the luteal phase progresses, estradiol rises again, reaching a second peak 5-7 days after ovulation. Decreasing levels of estradiol and progesterone in the late luteal phase result in endometrial breakdown and, eventually, menstruation (Muizzuddin et al., 2005).

Normal menstrual cycle begins at 11–14 years of age, with a normal cycle interval is 21–45 days, and the normal length of menstrual flow is seven days or less with an average cycle requiring no more than 3–6 pads or tampons per day (Diaz et al., 2006). Normal menstruation does include a haemostatic process involving primary platelet aggregation followed by secondary fibrin formation as well as fibrin clot modelling through fibrinolysis. Any deviation from this process can result in menorrhagia (Kouides and Kadir, 2007) (Figure 2.5).

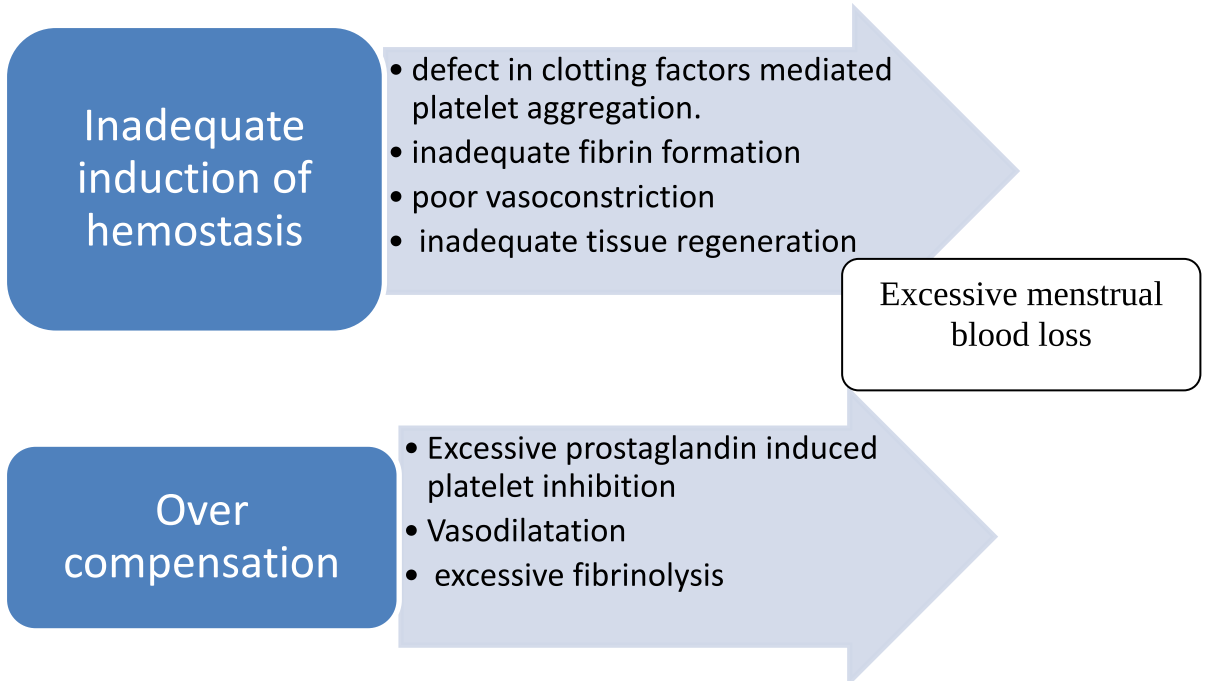

Figure 2.5 Pathophysiology of homeostasis during menstrual cycle leading to menorrhagia(129)

2.4.5.2 Assessment of menstrual blood loss

Menstruation and ovulation may be accompanied by significant bleeding resulting in limitation in performing daily activities as well as adverse effect on social functioning and quality of life. Menorrhagia is defined as a complaint of heavy cyclical menstrual bleeding occurring over several consecutive cycles (Royal College of Obstetricians and Gynaecologists, 1998). An objective definition of menorrhagia is menstrual bleeding of 80 ml or greater per menstruation (Hallberg et al., 1966).

Menstrual blood loss can be assessed using pictorial blood-assessment chart (PBAC) that relies on visual assessment and scoring of sanitary pad and tampon saturation (Higham et al., 1990). This method was developed in an attempt to create a simple non laboratory method of assessing menstrual blood loss that is more accurate than other simple methods such as tampon and pad counting and the weighing of such blood-stained materials. In addition to recording the number of the towels and tampons used, the degree to which individual items are soiled is also taken into consideration. In this method, women use special sheet to document the number of sanitary pads and/or tampons used each day (24 hour) based on the degree of saturation of the pads/tampons, and record the number and size of blood clots if present (Appendix 4). The total score are calculated by adding up the pad/tampons counts obtained from each day of the period. The scores assigned for tampons were “1” for each lightly stained tampon, “5” if moderately stained and “10” if it was completely saturated with blood. The towels were given ascending scores of “1”, “5” and “20”. Small and large clots scored “1” and “5”, respectively. PBAC score$\geq$ 100 were considered a heavy menstrual bleeding equivalent to more than 80 ml blood loss. The chart was found to have a sensitivity of 86% and a specificity of 89%, using scores used by the women. The sensitivity and specificity were 86% and 81%, respectively, using the gynaecologist's score (Higham et al., 1990). The test was shown to be an accurate tool in measuring menstrual blood loss (Zakherah et al., 2011).

2.4.5.3 Menorrhagia

Menorrhagia is a common gynaecological problem and result in seeking medical attention in 5–10% of women of reproductive age (Oehler and Rees, 2003). Causes of menorrhagia can vary from organic, anatomical and endocrine. Organic causes include genitourinary infections or organic dysfunction as hepatic or renal failure. Abnormal liver function will affect the synethesis of the clotting factors and impair hormone metabolism such as oestrogen. Abnormal endocrine fuction can lead to menorrhagia, especially anovulatory dysfunctional uterine bleeding among adolescent females due to immature hypothalamic–pituitary–ovarian axis. Anatomic causes for menorrhagia include uterine fibroids, endometrial polyps and hyperplasia. Other causes of increased menstrual bleeding include: the Intra Uterine Contraceptive Device (IUCD), corticosteroids, anticoagulants and chemotherapy (Warner et al., 2004).

Despite all the known causes of menorrhagia mentioned above, around 50% of those with objective menorrhagia had no uterine pathology when they were examined following hysterectomy for non-malignant reasons (Clarke et al., 1995). For such groups it is important to search for underlying bleeding disorders that can be presented as menorrhagia. Previous studies have found inherited bleeding disorders, mainly von Willebrand Disease (VWD), in 17% of patients with menorrhagia when defined by PBAC score more than 100

(Kadir et al., 1998), 20% of menorrhagia when defined based on the amount of blood loss (Edlund et al., 1996), and 10.7% of menorrhagia based on medical records (Dilley et al., 2001) . The prevalence of menorrhagia among women with bleeding disorders can vary based on the disease type, from 74% among women with VWD to 57% among carriers of haemophilia and 59% among women with factor XI deficiency (Kadir et al., 1999b). The mechanism of menorrhagia in women affected by bleeding disorders might be related to a defect in the formation of a platelet plug that is an important phase in regulating menstrual blood flow (Christiaens, 1996).

Diagnosis of menorrhagia have been an important element in the evaluation of women referred for the possible diagnosis of bleeding disorder and thus was incorporated into the bleeding score assessment system designed through the ISTH (Rodeghiero et al., 2010; Rydz and James, 2012).

Menorrhagia due to bleeding disorder is expected when there is a prolonged menstrual bleeding more than seven days duration, menstrual bleeding per cycle more than 80 ml, soaking one pad or tampon per hour, producing clots larger than one inch in diameter, blood soaked onto clothes or bed, or PBAC score of more than 100 or 150 (James, 2009; Zakherah et al., 2011). Those with a significant history of menstrual bleeding should be further screened for coagulation tests including complete blood count, PT, APTT, thrombin time, Von willebrand factor (VWF) antigen, factor VIII levels, platelet function analyses and FXIII level.

The initial step in the management of menorrhagia in women with bleeding disorders is through conservative or medical therapy in the form of hormonal therapy, such as combined oral contraceptive pills, or haemostatic therapy including antifibrinolytic (tranexamic acid and aminocaproic acid) and DDAVP or desmopressin, as well as replacement therapy with coagulation factors (Peyvandi et al., 2011b). Surgical management may be required in some cases, especially haemophilia carriers, and treatment varies from levonorgestrel-releasing IUD, endometrial ablation or hysterectomy (Sanders et al., 2012).

2.4.5.4 Ovulation bleeding:

Women with bleeding disorders are at risk of bleeding from ruptured ovarian follicles. At the time of ovulation, an ovum extruded from the follicle on the surface of the ovary into the peritoneal cavity. Usually, this process is not associated with significant bleeding (Reproductive endocrinology, 2001). However, women with bleeding disorders may have a higher amount of bleeding that presents in various forms; either into the peritoneal cavity in the form of mid cycle vaginal bleeding or pain (Mittelschmerz sign); bleeding to the residual follicle (the corpus luteum) resulting in a haemorrhagic ovarian cyst; or bleeding into the uterine broad ligament resulting in a retroperitoneal haematoma. Mittelschmerz sign in rare bleeding disorders was reported in a survey on 81 menstruating women with type 1 VWD were 49% reported mid-cycle pain (Kouides et al., 2000).

Haemorrhagic ovarian cysts, while less common than menorrhagia, have been observed in many case reports of involving women with bleeding disorders. In a case series of 136 women with VWD, nine (6.8%) had haemorrhagic ovarian cysts (Silwer, 1973). Lak et al reported a case series of women with FXIII deficiency, and four out of 20 women (20%) experienced ovarian bleeding, requiring hysterectomy in one of the cases (Lak et al., 2003).

The importance of recognising rare bleeding disorders in patients with haemorrhagic ovarian cysts lies in the need of providing adequate prophylaxis prior to surgical treatment of ovarian cysts and intra-abdominal bleeding, or manage those patients through non-surgical approach when possible. Oral contraceptives pills (OCP) have been used as a prophylaxis against the development of this type of bleeding by preventing ovulation (Payne et al., 2007).

2.4.5.5 Miscarriage

Recurrent miscarriage is defined as spontaneous loss of three or more consecutive pregnancies occurring before week 20 of pregnancy, and it affects 1% of women of reproductive age while the loss of two or more consecutive pregnancies affects approximately 5% of women (Rai and Regan, 2006). Recurrent miscarriage is attributed to various causes ranging from anatomical, genetic, infectious, endocrine, immune, and idiopathic. In a recent study on the aetiology of recurrent miscarriage, abnormal embryonic karyotype was considered the most common causes of recurrent miscarriages in their study cohort (Sugiura-Ogasawara et al., 2012). The risk of miscarriage is double when at least one member of a couple is a carrier of chromosomal anomalies. Therefore, karyotyping of both couples is a standard investigation for cases with recurrent miscarriage. The maternal age may also be a risk factor as there is an observed trends toward increased unbalanced structural anomalies in younger mothers and decreased viable autosomal trisomies in the older women (Grande et al., 2012). Uterine anatomical abnormalities are also within the common cause of miscarriages, mainly septate, bicornuate and didelphic uterus. Cervical anomalies due to damage from birth trauma or surgical intervention may also contribute into the aetiology of repeated pregnancy loss (Porcu et al., 2000). Endocrine anomalies are strongly related to recurrent miscarriages such as thyroid dysfunction, thyroid autoimmunity (Abalovich et al., 2002), polycystic ovarian syndrome (Homburg, 2008), and type one diabetes mellitus (Christiansen et al., 2008). Miscarriage can also be the outcome of atypical humoral or cellular immunological responses towards the embryo. Thrombophilia, both inherited and acquired, have been associated with risk of recurrent miscarriage (McNamee et al., 2012a). The most common example is antiphospholipid antibody syndrome seen in 15% of women with recurrent pregnancy loss and can be associated with a high risk of pregnancy loss up to 90% if no treatment was given during pregnancy (Rai et al., 1995). The mechanism of pregnancy loss in these cases were mainly through thrombosis of uteroplacental vasculature resulting in placental infarction, in addition to defect in endovascular trophoblast invasion (Sebire et al., 2002). Hereditary thrombophilia caused by mutations in the factor V and prothrombin gene, antithrombin as well as protein C and S deficiency are also known causes for recurrent miscarriages (Rodger et al., 2008).

Despite detailed evaluation, around 50% of cases are unexplained (Li et al., 2002). This makes recurrent miscarriage an emotional and physical challenge for any couple who experience the repeated loss of their offspring together with anxiety from the idea of losing further pregnancies due to miscarriage.

Pregnancy is associated with changes in the maternal coagulation and fibrinolytic system which is necessary for the development of uteroplacental circulation; a balance in these two systems is required to maintain pregnancy to prevent excessive fibrin deposition in placental vasculature and intravillous spaces, maintain fibrin polymerisation, and stabilise the placental basal plate (Buchholz and Thaler, 2003). This has led to speculations for two further causes for recurrent miscarriage; haemorrhagic and thrombotic.

Thrombotic disorders are more commonly associated with recurrent miscarriage, possibly through microthrombi formation in early placental vessels (Bick, 2000). These disorders include antiphospholipid syndrome; reduced activity of tissue plasminogen activator; increased PAI-1; deficiency of protein C, S; antithrombin III and heparin cofactor II(Bick and Hoppensteadt, 2005; Brenner, 2010). However, except for antiphospholipid syndrome, the association of most other thrombophilic disorders with recurrent miscarriage needs to be debated as most research conducted in this area are observational or case control studies involving small cohort of women (McNamee et al., 2012a, 2012b).

Bleeding disorders have also been described in small series to be associated with recurrent miscarriage, including prothrombin deficiency, reduced factor V, VII, X, XII and XIII, and afibrinogenemia (Jones et al., 2003; Bick and Hoppensteadt, 2005; Settin et al., 2011).

Women with congenital FXIII deficiency have been known to be at risk for recurrent early pregnancy loss (Rodeghiero et al., 1987; Inbal and Kenet, 2003; Asahina et al., 2007), especially in the presence of a history of severe bleeding diathesis as delayed umbilical bleeding and ICH (Burrows et al., 2000; Gmez-Garca et al., 2001; Meili, 2002). A study of 351 women with recurrent miscarriage found FXIII deficiency in one (0.3%) of the cases (Bick and Hoppensteadt, 2005).

Homozygous FXIII Val34Leu polymorphisms have been suggested to be associated with a high risk of early pregnancy loss (Dossenbach-Glaninger et al., 2003). In addition, Anwar et al (Anwar et al., 1999) suggested that Tyr204Phe polymorphism was more commonly seen among women with history of recurrent miscarriage; However, in a meta-analysis study, factor XIII Val34Leu and Tyr204Phe polymorphisms were not significantly associated with recurrent miscarriage (Sotiriadis et al., 2007). A more recent study of 100 Iranian women with history of at least two pregnancy losses found a significantly high prevalence of Tyr204Phe and Pro564Leu polymorphism among women with recurrent miscarriage while Val34Leu polymorphism was not significantly related to recurrent pregnancy loss (Jeddi-Tehrani et al., 2010). It is thought that FXIII may cross-link substrates, other than fibrin, during its role in maintaining pregnancy and that some heterozygous mutation may be associated with recurrent miscarriage by altering the structure of FXIII with no direct effect on FXIII level and activity.

In addition, the presence of other coagulation factor polymorphisms may have a synergistic effect on the role of FXIII polymorphism in inducing recurrent miscarriage. PAI-1 plays a major role in hypofibrinolysis and thrombotic complications. The PAI-1 gene expression is modulated by a 4G/5G polymorphism in the promoter region (Mikkola et al., 1997). Studies on arterial thrombotic tissues and pulmonary emboli concluded that PAI-1 polymorphism, when associated with FXIII Val34Leu polymorphism, can increase the risk of fibrinolysis impairment (Kohler, 2001). This is done through inhibiting the fibrinolytic system activity and increasing fibrin network resistance against fibrinolysis. Elevated PAI-1 concentration could result in defect in fibrinolysis and coagulation system and hence increase thrombosis, thus can initiate placental damage and thrombotic complication (Kohler, 2001). However, this was debated in a later study, comparing 63 women with recurrent miscarriage to a control group, and found only PAI-1- 4G polymorphism to be a risk factor for recurrent miscarriage, but no similar role of FXIII Val34Leu polymorphism.

The mechanism behind pregnancy loss in patients with FXIII deficiency is still not precisely known. It is believed that women with FXIII deficiency lack FXIII-A in the placental bed, resulting in early pregnancy loss due to poor formation of the cytotrophoblastic shell, and hence increasing the risk of placental detachment and eventual miscarriage within the first trimester (Asahina et al., 1998, 2000, 2007).

2.4.5.6 Antepartum and Postpartum Haemorrhage

Pregnancy and delivery represents one of the greatest haemostatic challenges to women, and an important cause of maternal mortality in many parts of the word. In the 2006–2008 UK Confidential Enquires into Maternal Death, obstetric haemorrhage was the fourth most common cause of direct maternal death. Many of the cases reviewed were considered to have been avoidable and 70% were secondary to substandard care (Wilkinson and Trustees and Medical Advisers, 2011). The risk of obstetric bleeding in women with bleeding disorders has only been addressed in the past few decades, representing a serious source of morbidity and mortality in these group of women due to lack of awareness for early diagnosis and management.

Antepartum haemorrhage (APH) is defined as vaginal bleeding occurring from 24 weeks of gestation. The incidence of APH ranges from 5.9 to 6.5 per 1000 with a very high perinatal mortality especially in placental abruption (119 per 1000 births) (Bhide and Thilaganathan, 2004; Giordano et al., 2010).

The prevalence of APH in women with FXIII deficiency is not clear. APH was observed in a case report of a woman with FXIII-A deficiency who experienced APH in her second pregnancy at 32 weeks of gestation. She was on FXIII prophylaxis, and her pregnancy progressed without adverse maternal and neonatal outcome (Gmez-Garca et al., 2001). Two pregnancies occurred in a woman with FXIII-A deficiency, not on prophylaxis who had severe APH due to placental abruption at 31st and 37th weeks of gestation (Mikkola et al., 1997).

Postpartum haemorrhage (PPH) is classified as primary or secondary PPH. Primary PPH is classically defined as a blood loss in the first 24 h after delivery with the amount of blood loss more than 500 mL to 1000 mL. Secondary PPH refers to excessive bleeding occurring between 24 h and 6 weeks post-delivery. Women with inherited bleeding disorders are at an increased risk of both primary and secondary PPH (Kadir et al., 2009). A large population-based study in Norway was done using medical records of more than 300,000 pregnant women to determine the prevalence and risk factors for severe PPH (> 1500 mL). The results found VWD, one of the common bleeding disorders, to be the second most common cause of severe PPH and second only to emergency caesarean delivery (Al-Zirqi et al., 2008). PPH have also been observed in few case reports and series of women with FXIII – A deficiency (Saito et al., 1990; Burrows et al., 2000; Ivaskevicius et al., 2010b) and FXIII-B deficiency (Ivaskevicius et al., 2010b).

Risk of PPH in women with FXIII deficiency can be reduced through three main approaches; providing prophylactic treatment to maintain stable haemostatic status, prevention and early management of uterine atony and delivery with minimal genital trauma. Prophylactic replacement therapy is given for at least 3–5 days after vaginal delivery or 5–7 days following caesarean section to maintain the required amount of clotting factor level and prevent primary and secondary PPH. The use of oral tranexamic acid for 3–4 days post vaginal delivery or 7–10 days following caesarean section can also be considered in some cases. However, this should be balanced against the risk of thrombosis associated with replacement therapy. Active management of third stage of labour using uterotonic agents had significantly reduced the incidence of uterine atony, a common cause of PPH (Begley et al., 1996). In case of PPH, an initial assessment and restoration of circulatory volume is needed either with fluid or blood transfusion, local causes should be excluded and replacement of the deficient clotting factor with monitoring of the factor levels should be performed in collaboration with haematologists (Kadir et al., 2009).

CHAPTER THREE

CONGENITAL FACTOR XIII DEFICIENCY IN WOMEN; A SYSTEMATIC REVIEW OF LITERATURE

3 CHAPTER THREE: CONGENITAL FACTOR XIII DEFICIENCY IN WOMEN; A SYSTEMATIC REVIEW OF LITERATURE

3.1 Introduction:

The first clinical report of FXIII deficiency was described in 1960 (Duckert et al., 1960); since then, more than 500 cases of FXIII deficiency have been identified worldwide with an incidence of one individual in 1-3 million (Ivaskevicius et al., 2007a; Muszbek et al., 2011a). Congenital FXIII deficiency is characterised by severe delayed spontaneous bleeding with normal coagulation screening tests.

Women are more vulnerable to manifest a bleeding disorder because they are at high risk to experience bleeding challenges during menstruation and child bearing. Women with FXIII deficiency often fail to produce the same levels of clotting factors as normal women, making them vulnerable to bleeding diathesis, delayed wound healing and adverse pregnancy outcome, mainly miscarriage.

Being a very rare condition, assessment for FXIII deficiency is not part of the routine evaluation tests for miscarriage unless there is a strong history of severe bleeding. Asahina *et al*, studied a case of FXIII deficiency with miscarriage and linked deficiency of FXIII with early pregnancy loss and implantation failure due to insufficient formation of extravillous cytotrophoblast (Asahina et al., 2000). For such women FXIII concentrate is the treatment of choice, either plasma-derived or recombinant when available. It is important to give the proper amount of FXIII concentrate with treatment intervals to achieve normal levels of FXIII during the gestational period (Asahina et al., 2007).

However, it is still unclear how to choose the minimum plasma level of FXIII needed to maintain pregnancy and prevent bleeding complications. Different guidelines provide different recommendations regarding the dose and interval of FXIII concentrate administered during pregnancy and labour and the level above which FXIII should be maintained to avoid adverse pregnancy outcome (Rodeghiero et al., 1987; Boda et al., 1989; Asahina et al., 2000, 2007; Burrows et al., 2000).

There is a paucity of data in the literature about women affected with FXIII deficiency due to the rarity this disorder. This systematic review explores the existing literature to include case reports and case series examining the obstetrics and gynaecological outcome in women with congenital FXIII deficiency.

3.2 Materials and Methods

3.2.1 Search strategy and inclusion criteria:

An electronic search was performed to identify the published literature on PUBMED, MEDLINE, EMBASE, Journals @OVID and CINAHL Plus databases using the following keyword combination: ‘congenital factor XIII deficiency’ AND ‘women OR Pregnancy’. The bibliographic references of all retrieved studies were further assessed for additional sources of clinical studies.

There were no time or language restrictions but clinical studies were only considered suitable if they were related to reproductive age women with factor XIII deficiency. FXIII deficiency was included if women had haemorrhagic diathesis (e.g. umbilical cord bleeding or intracranial haemorrhage) with or without a history of abnormal gynaecologic or obstetric outcome and in the presence of abnormal clot-solubility test or abnormal FXIII quantitative assay (FXIII activity < 70 IU/dL).

3.2.2 Study selection:

The initial search revealed 933 potentially relevant articles and the full selection process is presented in a flow diagram (Figure 3.1). After excluding articles on grounds of duplication and irrelevance to women or the lack of enough data on the course and outcome of the cases, 39 articles were included in this systematic review. These were 27 case reports (Ikkala et al., 1964; Fisher et al., 1966; Hamer and Rae, 1971; Girolami et al., 1986, 1977; Rodeghiero et al., 1987; Capellato et al., 1987; Boda et al., 1989; Kobayashi et al., 1990; Saito et al., 1990; Mikkola et al., 1997; Asahina et al., 1998, 2000; Cerenzia et al., 1999; Burrows et al., 2000; Gmez-Garca et al., 2001; Koseki et al., 2001; Meili, 2002; Padmanabhan et al., 2004; Rott et al., 2004; Lovejoy et al., 2006; Takahashi et al., 2007; Melo, 2008; Singh et al., 2008; Dargaud et al., 2008; Hanke et al., 2010; Chakravarty et al., 2012) and 12 case series (Burrows et al., 2000; Lak et al., 2003; Peyvandi et al., 2004; Bhattacharya et al., 2005; Medhaffar et al., 2006; Schroeder et al., 2007; Ivaskevicius et al., 2007b, 2010a, 2010b; Vijapurkar et al., 2009; Ichinose, 2012; Naderi et al., 2012) dating from 1964 to 2012. The papers were written in English, except five written in Spanish (Melo, 2008), Dutch (Meili, 2002), Italian (Cerenzia et al., 1999), Japanese (Takahashi et al., 2007), and French (Medhaffar et al., 2006).

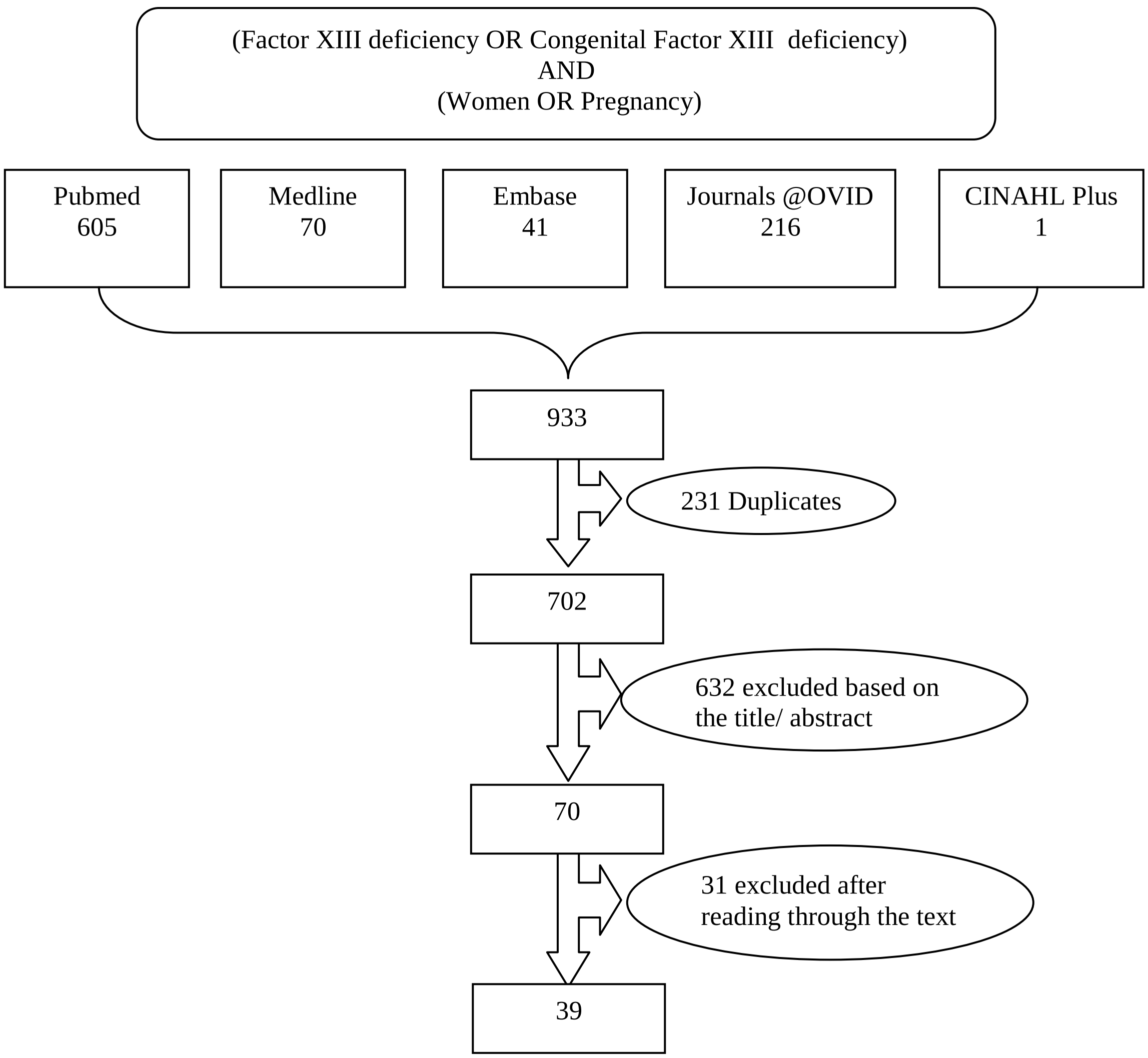

Figure 3.1 Flow diagram of study selection

3.2.3 Data extraction:

The following data were extracted: Author and year of publication; age at the time of reporting and age at diagnosis; the country of origin; gravidity and parity; history of bleeding episodes, including menorrhagia, and treatment received. For each pregnancy the following data were collected; bleeding during pregnancy and gestational age at the time of bleeding, miscarriage and gestational age at time of miscarriage, prophylactic treatment prior to and/or during pregnancy; mode of delivery; gestational age at delivery, presence of antenatal complications; occurrence of postpartum haemorrhage; other postpartum complications; and neonatal outcome.

3.3 Results:

A total of 121 women were identified from 39 articles. There were 27 case reports including 30 women (Appendix 1), and 12 case series including 104 women (Appendix 2). Two case series (Burrows et al., 2000; Ichinose, 2012) included 13 women who were also reported individually as case reports (Ikkala et al., 1964; Fisher et al., 1966; Hamer and Rae, 1971; Girolami et al., 1977, 1986; Capellato et al., 1987; Rodeghiero et al., 1987; Boda et al., 1989; Saito et al., 1990; Kobayashi et al., 1990; Koseki et al., 2001; Lovejoy et al., 2006). These 13 cases were included only once, making the total number of women in the review 121 women. Demographic and clinical data are presented in Table 3.1 and Table 3.2 while the bleeding symptoms in the case reports are in Appendix 3. FXIII-A deficiency was reported in 104 women and FXIII-B deficiency in 17 women. Table 3.3 presents a comparison of gynaecological bleeding and pregnancy outcome between women with FXIII-A and B deficiencies.

Table 3.1 Demographic and clinical variables of 121 women (192 pregnancies) with FXIII deficiency.

Variables		References
Age at time of case report, years ; median (range)	30 (13-52)	
Age at diagnosis, years ; median (range)	18 (0.5-35)	(Ikkala et al., 1964; Fisher et al., 1966; Saito et al., 1990; Padmanabhan et al., 2004; Melo, 2008; Ivaskevicius et al., 2010a, 2010b)
Consanguinity, n (%)	16/66 (24)	(Ikkala et al., 1964; Fisher et al., 1966; Saito et al., 1990; Padmanabhan et al., 2004; Melo, 2008; Ivaskevicius et al., 2010a, 2010b)
FXIII-A deficiency, n (%)	104 (86)	
FXIII-B deficiency, n (%)	17 (14)	(Girolami et al., 1977; Capellato et al., 1987; Saito et al., 1990; Burrows et al., 2000; Koseki et al., 2001; Rott et al., 2004; Lovejoy et al., 2006; Ivaskevicius et al., 2010b)

Table 3.2 Factor XIII activities and laboratory methods reported in the literature review

Variable	No (%)	References
FXIII activity <5 IU/dL, n (%)	64 (53)	(Ikkala et al., 1964; Girolami et al., 1977; Rodeghiero et al., 1987; Boda et al., 1989; Mikkola et al., 1997; Burrows et al., 2000; Gmez-Garca et al., 2001; Meili, 2002; Lak et al., 2003; Medhaffar et al., 2006; Ivaskevicius et al., 2007b; Dargaud et al., 2008; Vijapurkar et al., 2009; Naderi et al., 2012; Chakravarty et al., 2012)
FXIII 6-20 IU/ dL, n (%)	10 (8)	(Girolami et al., 1986; Capellato et al., 1987; Kobayashi et al., 1990; Asahina et al., 1998, 2000; Koseki et al., 2001; Peyvandi et al., 2004; Rott et al., 2004; Lovejoy et al., 2006; Dargaud et al., 2008)
FXIII 40-60 IU/ dL, n (%)	17 (14)	(Hanke et al., 2010; Ivaskevicius et al., 2010a)
FXIII activity not reported, n (%)	30 (25)	
Laboratory methods used for diagnosis of FXIII deficiency.		
• Urea clot solubility, n (%)	30 (25)	
• Immunoassays of FXIII A and B subunit, n (%)	42 (35)	
• Amonia release assay, n (%)	4 (3)	
• Genotype analysis, n (%)	40 (33)	
• Not specified, n (%)	5 (4)	

Table 3.3 Comparison of gynaecological bleeding and pregnancy outcome between women with FXIII-A and B deficiencies

Obstetric and Gynaecological bleeding symptoms	FXIII-B subunit deficiency (17 women, 13 pregnancies)	FXIII-A subunit deficiency (104 women, 179 pregnancies)
Menorrhagia	4/17 (13%)	27/104 (26%)
Ovulation bleeding	0/17 (0%)	10/104 (10%)
Miscarriage	2/13 (15%)	125/179 (70%)
APH	3/11* (27%)	2/54* (4%)
PPH	9/11 (82%)	7/54 (13%)

* Viable pregnancies

3.3.1 Gynaecological bleeding:

Menorrhagia (heavy menstrual bleeding, HMB) was reported in 31/121 (26%) women in this literature review, making it the second most common bleeding symptom (Ikkala et al., 1964; Girolami et al., 1977, 1986; Rodeghiero et al., 1987; Capellato et al., 1987; Boda et al., 1989; Burrows et al., 2000; Gmez-Garca et al., 2001; Lak et al., 2003; Peyvandi et al., 2004; Bhattacharya et al., 2005; Lovejoy et al., 2006; Ivaskevicius et al., 2010b, 2007b, 2010a; Singh et al., 2008). Umbilical bleeding was the commonest bleeding symptoms and reported in 33/121 (27%) women.

Severe ovulation bleeding as an intraperitoneal bleeding occurring at the time of ovulation was reported in 10/121 (8%) of women (Ikkala et al., 1964; Fisher et al., 1966; Meili, 2002; Lak et al., 2003; Melo, 2008; Singh et al., 2008; Chakravarty et al., 2012). In two women, it led to the diagnosis of FXIII deficiency (Ikkala et al., 1964; Meili, 2002). Eight of these women were not on prophylaxis and two were on irregular prophylaxis using FFP. Six women required urgent surgical interventions including two women who had hysterectomy and bilateral salpingo-oophorectomy (Ikkala et al., 1964; Lak et al., 2003). Blood transfusion of 2-6 units was reported in four women (Fisher et al., 1966; Melo, 2008; Singh et al., 2008; Chakravarty et al., 2012). Prophylaxis to prevent further ovulation bleeding was started in four women. Details of women with ovarian bleeding are presented in Table 3.2.

Table 3.4 Details of women with FXIII deficiency and ovulation bleeding

Author (Year)	FXIII activity	Was on regular prophylaxis	Clinical History	Management	Postoperative treatment and prophylaxis
Chakravarty† et al, 2012	Not specified	Irregular use FFP	Umbilical bleeding at birth, multiple bruises, ICH, pelvic and renal hematoma. Acute abdomen (ovarian haematoma) age 13 years	FFP (4 U) and Blood (3 U), Laproscopic blood clot removed (2 L)	Blood (2U), tranexamic acid (500mg) for 2 days. OCP
Singh et al, 2008	Not specified	Irregular use FFP	Umbilical bleeding at birth, multiple bruises, ICH, pelvic and renal hematoma. Oral and nasal bleeding. Acute abdomen (ovarian haematoma) age 13 years	FFP (4 U) and Blood (2 U), Laproscopic blood clot removed (2 L)	Blood (4U), FFP (6 U), FFP /4-6 weeks
Meili, 2002	Berichrom test <5 IU/dL	None	Four episodes of corpus luteum rupture with abdominal bleeding,	Laparotomy	FXIII concentrate 500 mg/month
Fisher, 1966	Plasma soluble in 5M urea and 1 % monochloroacetic acid.	None	Four hospital admission for pelvic haematoma. Nerve compression.	Blood transfusion	Not specified
Ikkala, 1964	Plasma soluble in 5 M urea and 1 % monochloroacetic acid.	None	Three consecutive severe intra-abdominal bleeding at age 27 years. No previous bleeding history	Blood (4 U). Hysterectomy and adenexial removal	Not specified
Melo, 2008	7.5 IU/dL	None	Recurrent haematurea. Rupture haemorrhagic ovarian cyst with haemoperitoneum (1.5L)	Cryoprecipitate (8U) and RBC unit (2 U)	cryoprecipitate 6 U / month
Lak et al, 2003††	Not specified	Not specified	Not specified	One had hysterectomy and salpingo-oophorectomy	

† Both case report by Chakravarty et al and Singh et al share lots of similarities in their case description as if it is the same woman. However, no reference to that had been made in the papers. †† case series including four women

3.3.2 Pregnancy outcome:

Among women included in this literature review, pregnancy was reported in 63/121 (52%) women and a total of 192 pregnancies were observed: 127/192 (66%) resulted in a miscarriage and 65/192 (34%) reached viability stage. A history of at least one miscarriage occurred in 42/63 (67%) women with history of pregnancy, while recurrent miscarriage, defined as three or more pregnancy loss, was reported in 16/63 (25%) women. The median number of miscarriages was five, (range1-13). Two miscarriages occurred between five and seven weeks of gestation (Cerenzia et al., 1999; Padmanabhan et al., 2004); 14 miscarriages occurred between 8 and 12 weeks of gestation (Boda et al., 1989; Kobayashi et al., 1990; Padmanabhan et al., 2004; Dargaud et al., 2008); and 11 miscarriages between 16 and 18 weeks of gestation (Boda et al., 1989; Gmez-Garca et al., 2001; Dargaud et al., 2008). The gestational age in the remaining 100 miscarriages was not reported.

Viability status (pregnancy progressed to 24 week of gestation or more) was reached in 65 pregnancies and 62 delivered a live babies (Fisher et al., 1966; Girolami et al., 1977; Rodeghiero et al., 1987; Capellato et al., 1987; Boda et al., 1989; Kobayashi et al., 1990; Asahina et al., 1998; Burrows et al., 2000; Gmez-Garca et al., 2001; Meili, 2002; Lak et al., 2003; Padmanabhan et al., 2004; Rott et al., 2004; Lovejoy et al., 2006; Takahashi et al., 2007; Melo, 2008; Ivaskevicius et al., 2010a, 2010b; Naderi et al., 2012). There was one intrauterine death (IUD) at 37 week of gestation due to placental abruption (Cerenzia et al., 1999), and two neonatal deaths after preterm delivery at 25 (Dargaud et al., 2008), and 28 weeks gestation (Gmez-Garca et al., 2001).

3.3.3 Bleeding complications during pregnancy:

Among 65 pregnancies reaching viability, bleeding during early pregnancy was reported in only one woman; vaginal bleeding occurred in both of her pregnancies at 6 and 7 week gestations, and she was then started on prophylactic therapy with 500IU/week of FXIII concentrate until successful delivery at term (Kobayashi et al., 1990; Asahina et al., 1998).

Antepartum haemorrhage (APH: bleeding after 24 weeks gestation) occurred in five pregnancies in four women. Two out of four women had FXIII-B deficiency (Girolami et al., 1977; Saito et al., 1990). Four out of five pregnancies with APH occurred while not prophylaxis therapy (Girolami et al., 1977; Saito et al., 1990; Abalovich et al., 2002). One woman was on FXIII prophylactic therapy with APH at 32 week of gestation (Gmez-Garca et al., 2001). All five pregnancies resulted in healthy live babies. Three pregnancies were delivered through emergency caesarean section (Saito et al., 1990; Abalovich et al., 2002).

3.3.4 Mode of delivery:

The mode of delivery was reported in 23 pregnancies including 12 vaginal deliveries (Kobayashi et al., 1990; Asahina et al., 1998; Cerenzia et al., 1999; Burrows et al., 2000; Meili, 2002; Padmanabhan et al., 2004; Takahashi et al., 2007; Dargaud et al., 2008) and 11 caesarean delivery (Fisher et al., 1966; Rodeghiero et al., 1987; Boda et al., 1989; Saito et al., 1990; Mikkola et al., 1997; Rott et al., 2004; Melo, 2008; Hanke et al., 2010; Ivaskevicius et al., 2010b). The indication for caesarean delivery was an emergency caesarean for APH in three pregnancies(Saito et al., 1990; Mikkola et al., 1997); cephalo-pelvic disproportion in two (Rodeghiero et al., 1987; Saito et al., 1990); and premature rupture of membrane in one (Rott et al., 2004). An elective caesarean delivery for breech presentation was performed in two (Hanke et al., 2010; Ivaskevicius et al., 2010b) The indication for the caesarean delivery was not reported in the remaining three pregnancies (Fisher et al., 1966; Boda et al., 1989; Melo, 2008).

3.3.5 Neonatal outcome:

There were 62 live newborns; 61 delivered between 37 and 39 weeks gestation, one baby, delivered at 32 weeks (Gmez-Garca et al., 2001). Only eight babies had records of the cord blood FXIII level measuring 30, 31, 32, 43, 45, 58 and 107 IU/dL, respectively (Boda et al., 1989; Kobayashi et al., 1990; Saito et al., 1990; Asahina et al., 2000; Gmez-Garca et al., 2001). Newborn weight was available for eight babies: the weight at birth ranged from 3000-3142gms in seven babies (Fisher et al., 1966; Rodeghiero et al., 1987; Boda et al., 1989; Kobayashi et al., 1990; Asahina et al., 1998; Burrows et al., 2000), with one small for date weigh 2400 gm. There was one still birth at 37 week weighing 2400gm (23). There were also two neonatal deaths; one at 25 weeks and baby weighed 770 gm died few days later due to intracranial haemorrhage (Dargaud et al., 2008). The other neonatal death resulted from premature delivery at 28 weeks (Gmez-Garca et al., 2001).

3.3.6 Postpartum haemorrhage (PPH):

Primary PPH, was reported in 16/65 (25%) pregnancies that reached viability stage in 12 women. Five women had FXIII-B deficiency (Girolami et al., 1977; Capellato et al., 1987; Saito et al., 1990; Burrows et al., 2000). Eight pregnancies had PPH while not on prophylaxis therapy (Girolami et al., 1977, 1986; Capellato et al., 1987; Saito et al., 1990). Treatment of PPH was only described in two cases; the first case required concentrated red cells, FFP and laparotomy for PPH (Saito et al., 1990), the second case required whole blood transfusion (Girolami et al., 1977). Six women with PPH were reported as part of two case series, but no further details were available(Burrows et al., 2000; Ivaskevicius et al., 2010a). No detail about the estimated blood loss during delivery or information about secondary PPH was available.

3.3.7 Prophylaxis during pregnancy:

Prophylactic treatment was not given in 136 pregnancies. In 45 pregnancies, the mother received prophylactic replacement therapy. Information regarding the use of prophylactic therapy was not available in 11 pregnancies. Among the 136 pregnancies without prophylactic therapy, 124 (91%) resulted in miscarriage and only 12 progressed to viability stage (Girolami et al., 1977, 1986; Saito et al., 1990; Mikkola et al., 1997; Cerenzia et al., 1999; Gmez-Garca et al., 2001). Bleeding following miscarriage occurred in two women without prophylactic therapy (Girolami et al., 1977; Dargaud et al., 2008); one woman developed disseminated intravascular coagulation following evacuation of retained product of conception and was treated with FFP, four units of red blood cells, and uterine artery embolisation (Dargaud et al., 2008).

Twelve pregnancies reached viability stage without prophylaxis (Girolami et al., 1977, 1986; Saito et al., 1990; Mikkola et al., 1997; Cerenzia et al., 1999; Gmez-Garca et al., 2001) occurred in three women with FXIII-A deficiency with FXIII Activity less than 1, 3 and 10 IU/dL respectively, and six woman with FXIII-B deficiency (FXIII activity 1, 3, 10, 10, 10 and 24 IU/dL). Among these 12 pregnancies, APH occurred in three and PPH in eight pregnancies. Premature delivery at 28 weeks gestation resulting in neonatal death also occurred in one pregnancy without any bleeding complications (Gmez-Garca et al., 2001).

Of the 45 pregnancies on prophylaxis, 40 (89%) delivered live babies (Fisher et al., 1966; Rodeghiero et al., 1987; Boda et al., 1989; Kobayashi et al., 1990; Asahina et al., 1998; Burrows et al., 2000; Gmez-Garca et al., 2001; Meili, 2002; Lak et al., 2003; Padmanabhan et al., 2004; Rott et al., 2004; Takahashi et al., 2007; Melo, 2008; Ivaskevicius et al., 2010b; Naderi et al., 2012), one IUD at 37 week of gestation due to placental abruption (Cerenzia et al., 1999); one neonatal death due to intracranial haemorrhage following preterm delivery after premature rupture of membrane at 25th week (Dargaud et al., 2008); and three (7%) resulted in a miscarriage at 7,9 and 11 weeks gestation (Cerenzia et al., 1999; Asahina et al., 2000; Padmanabhan et al., 2004). Data on the use of prophylactic therapy was not available in nine pregnancies, all resulting in live births (Burrows et al., 2000; Hanke et al., 2010).

FXIII concentrate was the most common prophylactic treatment used in 33 pregnancies (Rodeghiero et al., 1987; Kobayashi et al., 1990; Asahina et al., 1998, 2000; Cerenzia et al., 1999; Burrows et al., 2000; Gmez-Garca et al., 2001; Meili, 2002; Rott et al., 2004; Takahashi et al., 2007; Dargaud et al., 2008; Ivaskevicius et al., 2010b; Naderi et al., 2012); the dose varied between 500 U every one to four weeks in four pregnancies (Rodeghiero et al., 1987; Kobayashi et al., 1990; Asahina et al., 1998; Burrows et al., 2000). The dose was increased in two pregnancies from 500 U to 750 U every two weeks at the 22nd week of gestation (Meili, 2002), and to 1250 U per week at 32 weeks of gestation(Takahashi et al., 2007). A dose of 10 U /kg was given every four weeks before pregnancy and then every two weeks during pregnancy in 17 pregnancies (Naderi et al., 2012). One woman with FXIII-B deficiency received 2500 U prior to caesarean delivery (Ivaskevicius et al., 2010b).

Cryoprecipitate was used in five pregnancies in a dose of 1-2 U every two weeks(Padmanabhan et al., 2004) and in a dose of 6-8 U per month (Lak et al., 2003; Padmanabhan et al., 2004). Human blood banked plasma was used in two pregnancies in dose of 300-400 ml every 10 days (Fisher et al., 1966; Boda et al., 1989) and FFP was used in two pregnancies in a dose of 300-450 ml every two weeks (Rodeghiero et al., 1987; Boda et al., 1989). Plasma FXIII level was kept in a range of 3-70 IU/dL with a median of 12 IU/dL during pregnancy. During labour and delivery, FXIII level was maintained at a median of 35 IU/dL (range 19-62 IU/dL) (Boda et al., 1989; Kobayashi et al., 1990; Asahina et al., 1998, 2000; Burrows et al., 2000; Rott et al., 2004; Takahashi et al., 2007; Hanke et al., 2010).

Two women received FXIII replacement therapy for primary infertility management: one woman with a two year history of primary infertility became pregnant two months after receiving 300-450 ml FFP every two weeks, and had a further pregnancy while on 500U FXIII concentrate every three weeks (Rodeghiero et al., 1987); the second woman had a FXIII activity of 50 IU/dL and a history of primary infertility with five unsuccessful attempts of IVF- this woman was treated with 2500 U FXIII every 10 days prior to and during her 6^{th} IVF attempt and a successful pregnancy resulted in a live baby (Rott et al., 2004).

3.4 Discussion:

Heavy menstrual bleeding is a common bleeding symptom in women with inherited bleeding disorders. In two previous case series, heavy menstrual bleeding was reported in 35% (Lak et al., 2003) and 64% (Burrows et al., 2000) women with congenital FXIII deficiency. In this review, menorrhagia was reported in 28% of women. However, this is most likely to be an underestimate as most case reports did not provide information on menstrual bleeding. In addition, due to the genetic nature of this condition, many women from the same family can be affected and have heavy menstrual bleeding. Such heavy periods may be viewed by women in these families as normal therefore leading to under reporting of menstrual problems.

Women with FXIII deficiency appear to be at an increase risk of significant ovulation bleeding leading to acute abdomen requiring surgical intervention, pelvic haematoma or haemorrhagic ovarian cysts (Inbal and Kenet, 2003). In a case series of 20 women with FXIII deficiency, 20% had a history of ovulatory bleeding (Lak et al., 2003). In this systematic review, severe ovulation bleeding leading to haemo-peritoneum occurred in 8% of the women. Management of such ovarian bleeding in the presence of FXIII deficiency poses additional challenges. There is a risk of further bleeding during surgery, which may require oophorectomy to control the bleeding, thus compromising the patient future fertility. Prevention of such bleeding by suppression of ovulation, and a conservative approach when the bleeding occurs, are the best option, as repeated haemorrhagic cysts and surgical removal of these cysts can cause ovarian damage (Ho et al., 2002; Ragni et al., 2005; Duru et al., 2007).

Women with FXIII deficiency have a significant risk of recurrent pregnancy loss. In this systematic review, 66% of the pregnancies resulted in a miscarriage. Animal studies have shown that mice with homozygous FXIII deficiency are more vulnerable to develop intrauterine bleeding and miscarriages. In a study of mice with homozygous FXIII-A deficiency, the histological examination of the uterine tissue showed blood pooling in the uterine cavity adjacent to the placenta and not in the amniotic cavity or the fetus. On the other hand, an intact placenta or uterus, was shown to be associated with a normal development of fetus even in homozygous mice. These findings suggest a maternal source of haemorrhage due to vascular breakage in the placenta, and that FXIII is crucial for the prevention of intrauterine bleeding and maintenance of pregnancy (Koseki-Kuno et al., 2003).

Although there is a significant increase in the risk of miscarriage (Kobayashi et al., 1990; Asahina et al., 1998), not all pregnancies in women with congenital FXIII deficiency result in a pregnancy loss. In this systematic review, 12 pregnancies in nine women progressed to viability stage and 11 delivered a live baby at term without receiving any replacement therapy prior or during pregnancy. However, three of the pregnancies were associated with APH (Girolami et al., 1977; Saito et al., 1990; Mikkola et al., 1997) and one had preterm delivery lead to a neonatal death (Gmez-Garca et al., 2001). There was also PPH in eight pregnancies (Girolami et al., 1977, 1986; Capellato et al., 1987; Saito et al., 1990). It was noted that six of these nine women, not on prophylaxis, were cases with FXIII-B deficiency. Clinical features of cases suffering from congenital FXIII-B deficiency are usually of milder course with less risk of severe bleeding compared to those with FXIII-A deficiency (Ichinose, 2001, 2012). Women with FXIII-B deficiency are able to conceive and progress to deliver live babies, despite the lack of replacement therapy (Ichinose, 2012). This may be explained by the presence of some residual FXIII activity. However, almost all of these women developed APH or PPH as well as post surgical bleeding. This was highlighted in this systematic review as the miscarriage rate (15%) was not higher than what is reported in the general population. However there was a higher frequency of both APH (27%) and PPH (82%) among these women.

This suggests that while FXIII activity in women with FXIII-B deficiency, reduces the chance of early pregnancy loss and spontaneous bleeding, is not enough to prevent bleeding complications during haemostatic challenges.

Three pregnancies resulted in a miscarriage despite being on prophylactic therapy during pregnancy. In these cases, low dose of treatment and/or missing prophylaxis have been reported as a possible cause for this adverse pregnancy outcome (Cerenzia et al., 1999; Asahina et al., 2000; Padmanabhan et al., 2004).

The exact prevalence of PPH among women with congenital FXIII deficiency is unclear. PPH is believed to occur less frequently in FXIII deficiency compared to other bleeding disorders (Peyvandi et al., 2011b), possibly because most women during pregnancy and delivery are receiving prophylactic therapy. FXIII concentrate has a long half life of 10-14 days. Thus, its protective effect extends to the postpartum period. In this systematic review, 25% of the deliveries were complicated with PPH; this is much higher than the prevalence of 8-10% reported in the general population (Carroli et al., 2008).

The finding of this review confirms that successful pregnancies in women with FXIII deficiency are generally only achieved with replacement therapy throughout pregnancy. However, it is not clear what level of FXIII is required for a successful pregnancy and safe delivery. Maintaining a FXIII level higher than 10-20 IU/dL has been recommended by some authorities (Peyvandi et al., 2011a). The United Kingdom Haemophilia Centre Doctors' Organisation(UKHCDO) recommends haemostatic cover with FXIII concentrate given monthly to all girls from the time of diagnosis and continued throughout pregnancy, aiming to keep the FXIII level >3 IU/dL (Bolton Maggs et al., 2004). In this systematic review, among 41 pregnant women on prophylaxis treatment reaching viability stage, the plasma FXIII level was maintained at a median of 12 IU/dL (range 3 to70 IU/dL) during pregnancy, and a median of 35 IU/dL (range 19-62 IU/dL) during labour and delivery. FXIII concentrate is the recommended treatment option and was the most common type of prophylactic therapy used during pregnancy and labour. Other treatment options include FFP and cryoprecipitate. There is no consensus on the optimal dose of FXIII concentrate but a dose of 250 IU weekly early in pregnancy until 23rd week, increased thereafter to 500 IU per week is recommended and widely used. For labour and delivery, a booster dose of 1000 IU is recommended to prevent PPH (Asahina et al., 2007; Peyvandi et al., 2011a).

Newborns of mothers with FXIII deficiency are at risk of being born preterm as a consequence to placental abruption. In a recent study among women with normal coagulation, perinatal mortality in pregnancies complicated with placental abruption was 102 compared to 6 per 1,000 births in those without abruption, mainly due to preterm delivery that occurred in 28% of cases (Ananth and VanderWeele, 2011).

FXIII replacement therapy during pregnancy can reduce the risk of placental abruption in women with FXIII deficiency. Therefore, these mothers should be managed in collaboration with a haemophilia centre with facilities to provide the haemostatic cover and regular monitoring of factor levels. In addition, obstetric management should include identification of any additional risk factor for preterm labour with regular surveillance and implementation of appropriate measure to improve outcome. Pregnancy should be managed in a unit with tertiary neonatal facilities with a facility to care for preterm babies.

FXIII deficiency is an autosomal recessive disorder. Thus, the baby has a 50% chance of being heterozygous for FXIII deficiency. Individuals with heterozygous FXIII deficiency have a residual FXIII activity around 30–60% and are usually asymptomatic with no spontaneous bleeding. However, delayed bleeding may develop under special conditions such as post surgery or dental extraction or following physical trauma. Therefore, heterozygote neonates are potentially at risk of bleeding during the process of labour and delivery, mainly head bleeding seen in traumatic delivery. In communities with consanguineous marriage, the neonate may be affected (homozygote) with FXIII deficiency with significant risk of bleeding, the commonest being umbilical bleeding (Anwar and Miloszewski, 1999; Anwar et al., 2002; Karimi, 2009); thus, partner screening should be considered in such communities. No specific study was found on the prevalence of intracranial haemorrhage among neonates with FXIII deficiency and there are no data or recommendations on the mode of delivery (Bolton Maggs et al., 2004). intracranial haemorrhage is more common among neonates with a history of prolonged labour and delivery using forceps or vacuum extraction, while caesarean delivery carries the lowest risk of intracranial haemorrhage in neonates in general and those affected with severe bleeding disorders such as haemophilia (Rooks et al., 2008). Therefore each case should be assessed individually by a multidisciplinary group including the mother, and prolonged, difficult deliveries should be avoided with early referral for caesarean delivery to minimise the risk. A planned caesarean delivery is increasingly used for delivery of babies affected with inherited bleeding disorders (Chi and Kadir, 2012) and should be considered if there is any additional risk for intracranial haemorrhage or if the labour is expected to be prolonged or difficult.

FXIII plays an important role in implantation that includes a complex interface between uterus and blastocyst through the action of activated FXIII on cross linking fibrin, fibrinogen and fibronectin which is essential in placental attachment to uterine tissue. FXIII-A has been detected within the extracellular spaces of extravillous cytotrophoblasts, as well as the Nitabuch's layer adjacent to the cytotrophoblastic shell (Kobayashi et al., 1999). It is believed that women with FXIII deficiency lack FXIII in placental bed resulting in failure of implantation and very early pregnancy loss due to poor formation of the cytotrophoblastic shell (Asahina et al., 1998, 2000, 2007). In this systematic review, two women with primary infertility, and repeated IVF failure, were able to have three pregnancies following administration of FXIII replacement therapy. The role of FXIII in the process of implantation and early pregnancy development is not well studied. Studies are required to assess the role of systematic and local (placental bed) level of FXIII in the process of implantation, and whether replacement and normalisation of FXIII can improve outcome in patient with implantation failure and early pregnancy losses.

In conclusion, this systematic review indicates that women with congenital FXIII deficiency suffer significant bleeding complications. However, we acknowledge the unavoidable limitation of this review as the clinical data varied markedly in content and quality between the reports and that reporting bias may have favoured descriptions of adverse gynaecological and obstetric events.

Menorrhagia and ovulation bleeding are common gynaecological problems and possibly more prevalent than reported. Pregnancies in women with FXIII deficiency have a significant risk of miscarriage, placental abruption, preterm delivery and PPH if not treated. Therefore, replacement therapy is essential to achieve successful pregnancy and to reduce the risk of bleeding during pregnancy, preterm delivery and PPH. The treatment of choice is FXIII concentrate, aiming to keep FXIII level between 3-10 IU/dL. In pregnancy, the dose is measured to keep FXIII level of > 10 IU/dL. For delivery, a booster dose may be necessary, especially if the delivery is operative to prevent PPH. Increased awareness among clinicians and a multidisciplinary approach is essential when managing the gynaecological and obstetrical issues affecting women with FXIII deficiency.

CHAPTER FOUR

FXIII LEVELS AND ITS CHANGES IN PREGNANCY AND IMMEDIATE POSTPARTUM PERIOD

4 CHAPTER FOUR: FXIII LEVELS DURING PREGNANCY AND IMMEDIATE POSTPARTUM PERIOD

4.1 Introduction:

Factor XIII (FXIII), fibrin stabilising factor, circulates in plasma as a heterodimer composed of two catalytic A-subunits and two carrier B-subunits (Muszbek et al., 1996). The A-subunits are produced by the haematopoietic cells (megakaryocyte and monocyte/macrophage) in the bone marrow whereas the B-subunits are produced in the liver (Wölpl et al., 1987). The main function of FXIII is in haemostasis, wound healing and the maintenance of pregnancy (Board et al., 1993; Anwar and Miloszewski, 1999). Variations in FXIII level can be attributed to many physiological and pathological factors. Significantly higher levels of the FXIII A subunit have been found among women and smokers, and there was an effect of age (Ariëns et al., 1999). The same study found FXIII B-subunit antigen and FXIII activity levels to be significantly correlated with the level of fibrinogen (Ariëns et al., 1999). The FXIII level has also shown to be increased in non–insulin-dependent diabetic patients with micro-angiopathy (Kłoczko et al., 1986).

Normal pregnancy is associated with significant changes in coagulation parameters (Franchini, 2006). A recent study found a marked increase in fibrinogen level and coagulation factors VII, VIII, and IX during pregnancy, delivery and postpartum; factors II, V, X, XI, XII, protein C and antithrombin, remained largely unchanged; while protein S activity decreased substantially (Szecsi et al., 2010). Changes in FXIII level during pregnancy have been assessed in very few studies and these contained small numbers of samples. These studies show a decrease in FXIII during the third trimester, however the minimum level required for fetal implantation and maintaining haemostasis during pregnancy are unknown. Furthermore the reference range of FXIII in pregnant women during the three trimesters of pregnancy has yet to be established (Rodeghiero et al., 1987; Boda et al., 1989; Burrows et al., 2000; Asahina et al., 2007).

The aim of this study was to establish the reference ranges for FXIII level during pregnancy and immediate puerperium and to assess changes in the level of FXIII during the three trimesters of a normal uncomplicated pregnancy. The relationship between the level of FXIII during pregnancy and the demographics of the women was also investigated.

4.2 Materials and Methods:

4.2.1 Study design:

This is a cross sectional study of 376 pregnant women, who attended the antenatal clinic at the Royal Free Hospital in London from October 2011 to July 2012. The study was approved by the hospital ethics committee and informed consent was obtained from participating women. Only women with a normal, uncomplicated pregnancy were included in the study. Women on anticoagulant or non-steroidal anti-inflammatory therapy and with personal or family history of bleeding disorders, or with any obstetric complications such as pre eclampsia and intrauterine growth retardation were excluded from the study.

Demographic data were obtained by direct interview with the women and from the obstetric case notes. These data included: age; maternal weight at the time of the blood sample, smoking status, previous obstetric history (number of pregnancies; previous miscarriages, history of gestational hypertension and history of previous intra-uterine growth retardation) and gestational age at the time of taking the blood sample. The obstetric notes were also reviewed after delivery for any obstetric complications subsequent to the blood sample, sex of the newborn, birth weight and gestational age and estimated blood loss (EBL) at delivery. Primary postpartum haemorrhage (PPH) was defined as blood loss in the first 24 hour after delivery with an estimated blood loss of more than 500 ml for normal vaginal deliveries, or more than 1000 ml for caesarean sections (RCOG, 2009).

The pregnancy duration was calculated based on the 1st trimester ultrasound determination. This is performed routinely for all pregnant women between11-13^{+6} weeks of gestation and gestational age is defined based on the crown-rump length. For the purpose of this analysis, pregnant women are divided into three groups based on their gestational age; first trimester group (gestational age 0-12 weeks, n=116), second trimester group (gestational age 13-28 weeks, n=132), third trimester group (gestational age 29-42 weeks, n=128) and immediate puerperium or postnatal (0-3 days post-delivery, n=30).

Sample numbers were chosen based on the International Federation of Clinical Chemistry (IFCC) recommendation to use a minimum sample size of 120 for calculating reference values (Horowitz et al., 2008). Blood samples were also collected from 25, age matched, healthy non-pregnant women as a control group.

In addition, a longitudinal study of 26 pregnant women was performed from the same study population who were followed up throughout pregnancy to measure FXIII activity during 1st, 2nd, and 3rd trimester of pregnancy.

4.2.2 Laboratory Methods:

After obtaining written informed consent, blood samples were obtained between 11:00 a.m. and 2:00 p.m. after a resting period of 10 minutes. Venous blood samples were collected by clean venepuncture, with minimal stasis, into citrate (0.105 mol/L) Vacutainers™ (BD Diagnostics, Oxford, UK), with a ratio of one part anticoagulant to 9 parts whole blood. Within one hour of collection platelet poor plasma (PPP) was prepared by double centrifugation at ambient temperature (2000g for 10 minutes) and aliquots of PPP were frozen to -80ºC. On the day of assay samples were thawed to 37ºC.

Factor XIII activity was determined using a chromogenic ammonia release assay (Berichrom® FXIII assay; Siemens Healthcare Diagnostics, Marburg, Germany) performed on a CS-5100 coagulation analyser (Sysmex UK Ltd, Milton Keynes) as described by Lawrie *et al* (Lawrie et al., 2010). FXIII potencies were calculated relative to Standard Human Plasma (SHP, Siemens Healthcare Diagnostics). The manufacturer's stated normal reference range was 70-140 IU/dL (Figure 4.1).

4.2.3 Statistical Analysis:

Descriptive statistics were performed for the demographic data and continuous data were presented as mean and standard deviation (SD). Variation in the mean FXIII levels between variable studied groups was assessed using unpaired student t-test. The longitudinal study on FXIII level changes during pregnancy was studied using paired student t-test. Data were analysed using SPSS statistical package, version 20.0 (SPSS Inc., Chicago, IL, USA). Level of significance was set at $p \leq 0.05$.

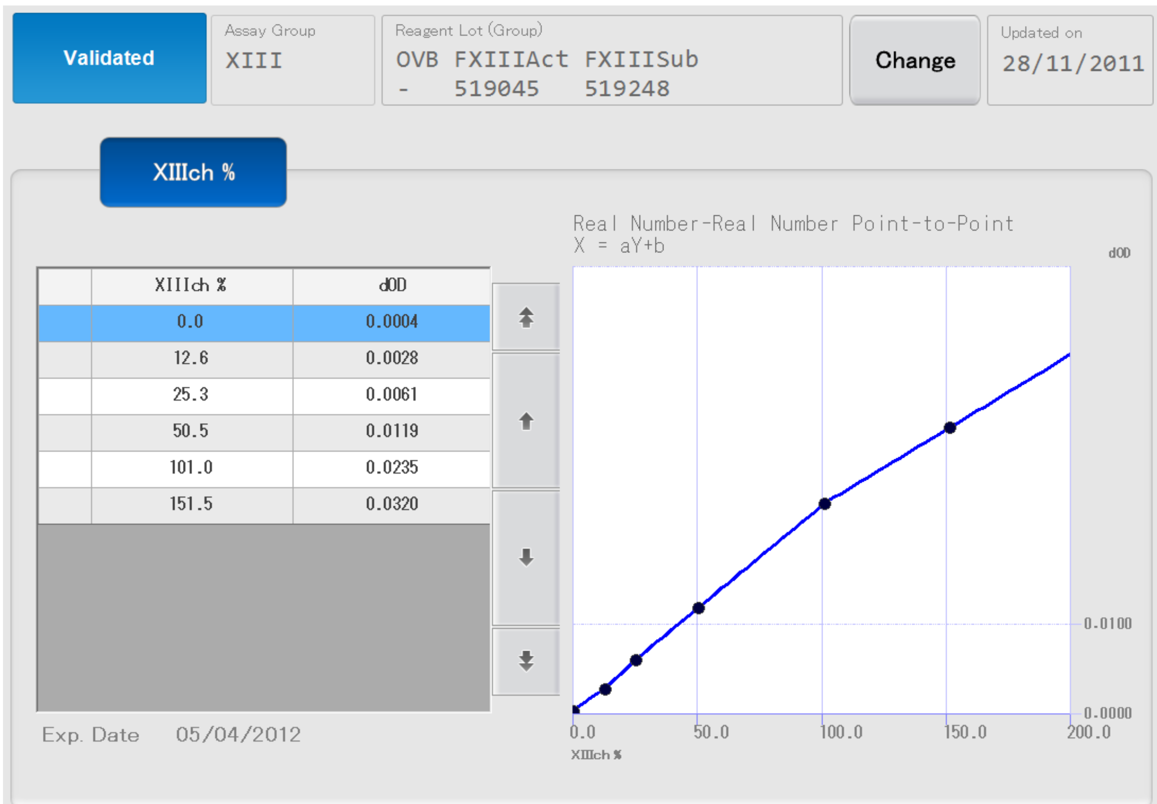

Figure 4.1 Example of a Berichrom® F XIII assay Standard curve

4.3 Results:

The demographic information of the women is presented in Table 4.1. The median age was 31 years (range 19 to 46 years). Only seven (2%) of the women were smokers. There were 116 women in the 1st trimester, 132 women in the 2nd trimester, 128 women in the 3rd trimester of pregnancy, and 30 women in the postnatal period.

FXIII activity was normally distributed throughout the gestational age and within each of the gestational age range groups (Figure 4.2, Figure 4.3, Figure 4.4). The mean ± SD FXIII activity was 112 ±29 IU/dL during the first trimester, 96 ± 26 IU/dL during the second trimester, 83± 21 IU/dL during the third trimester, 90 ± 19 IU/dL during postnatal period, and 113± 26 IU/dL among control group. FXIII range (Mean ± 2 SD) for the first trimester was 55 to 169 IU/dL (six results less than 70 IU/dL), for the second trimester 45 to 147 IU/dL (15 results less than 70 IU/dL), for the third trimester 42 to 125 IU/dL (34 results less than 70 IU/dL), for postnatal period 61 to 137 IU/dL (Five results less than 70 IU/dL) and for control group 61 to 165 IU/dL (no results were below 70 IU/dL) as seen in Table 4.2, Figure 4.5 Figure 4.6 and Figure 4.7.

Table 4.1. The distribution of women age, weight, gravid, gestational age and smoking status among all three trimesters of pregnancy

	1ST Trimester	2ND Trimester	3RD Trimester	TOTAL
Women included	n = 116	n = 132	n = 128	n = 376
Gravida				
Unknown	19 (16.4)	7 (5.3)	14 (10.9)	40 (10.6)
1	29 (25.0)	50 (37.9)	36 (28.1)	115 (30.6)
2	38 (32.8)	38 (28.8)	40 (31.3)	116 (30.9)
3	14 (12.1)	16 (12.1)	19 (14.8)	49 (13.0)
4+	16 (13.8)	21 (15.9)	19 (14.8)	56 (14.9)
Miscarriages				
Unknown	19 (16.4)	7 (5.3)	14 (10.9)	40 (10.6)
0	73 (62.9)	90 (68.2)	81 (63.3)	244 (64.9)
1	14 (12.1)	23 (17.4)	27 (21.1)	64 (17.0)
2	6 (5.2)	8 (6.1)	3 (2.3)	17 (4.5)
3+	4 (3.4)	4 (3.0)	3 (2.3)	11 (2.9)
Para				
Unknown	19 (16.4)	7 (5.3)	14 (10.9)	40 (10.6)
0	41 (35.3)	66 (50.0)	53 (41.4)	160 (42.6)
1	36 (31.0)	37 (28.0)	39 (30.5)	112 (29.8)
2	16 (13.8)	15 (11.4)	9 (9.0)	40 (10.6)
3+	4 (3.4)	7 (5.3)	13 (10.2)	24 (6.4)
Smoker				
Unknown	6 (5.2)	5 (3.8)	6 (4.7)	17 (4.5)
No	108 (93.1)	123 (93.2)	121 (94.5)	352 (93.6)
Yes	2 (1.7)	4 (3.0)	1 (0.8)	7 (1.9)
Gestation (wks); Median (range)	10 (7, 14)	25 (13, 29)	34 (13, 39)	26 (7, 39)
Age (years); Median (range)	31 (19, 42) n=103	31 (19, 46)	31 (20, 46) n=126	31 (19, 46) n=361
Body weight (kg); Median (range)	63.5 (43, 122) n=82	68.5 (43, 180) n=78	74 (50, 119) n=93	70 (43, 180) n=253

Unless otherwise stated the numbers given in parenthesis indicate the percentage of that group

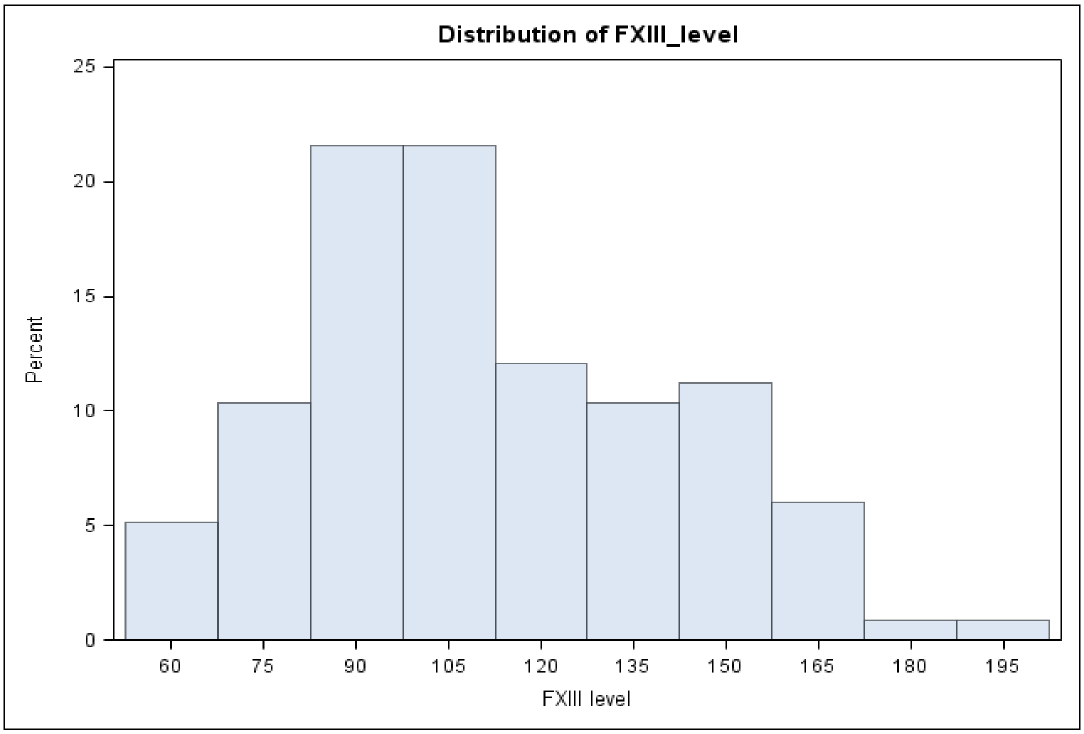

Figure 4.2 The distribution of FXIII activity (IU/dL) during first trimester of pregnancy

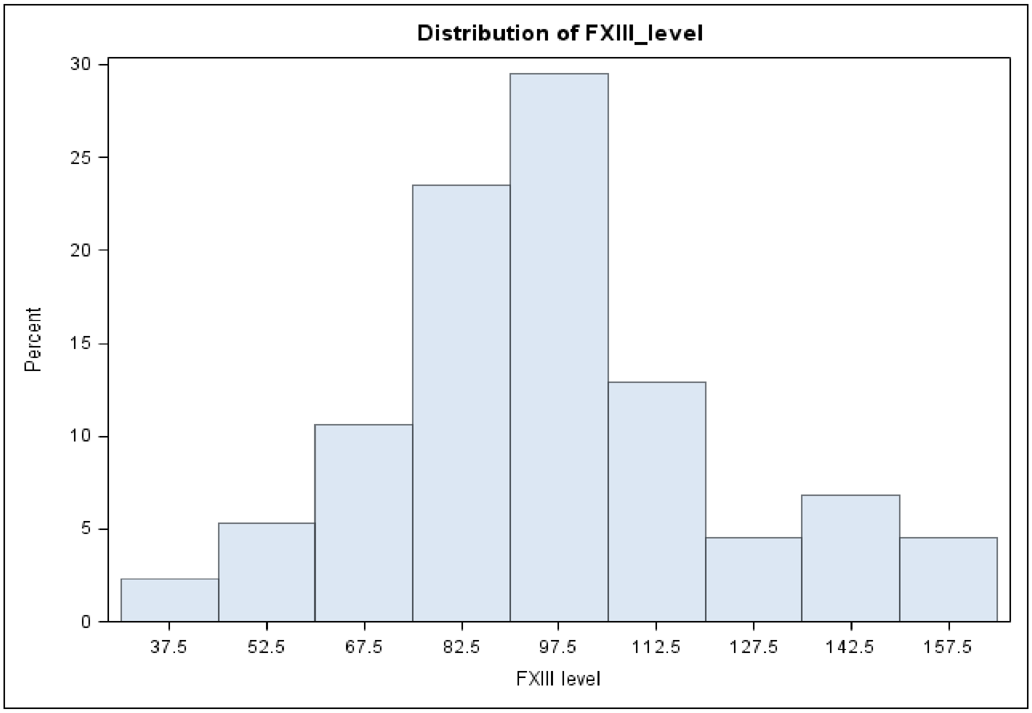

Figure 4.3 Distribution of factor XIII activity (IU/dL) in the second trimester

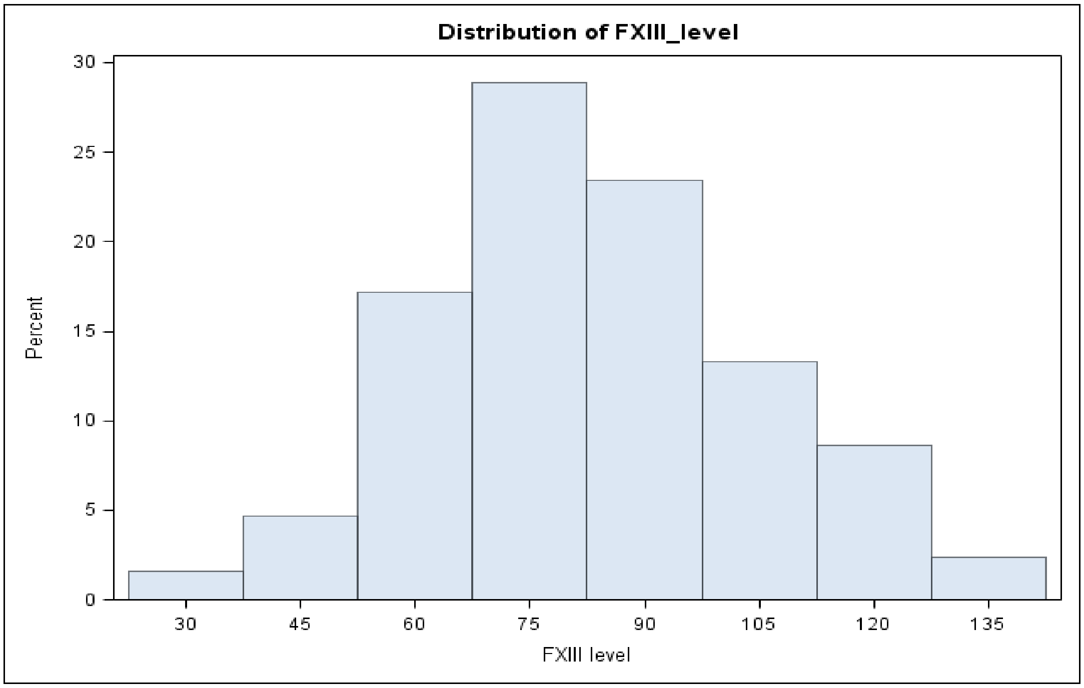

Figure 4.4 Distribution of Factor XIII activity (IU/dL) in the third trimester

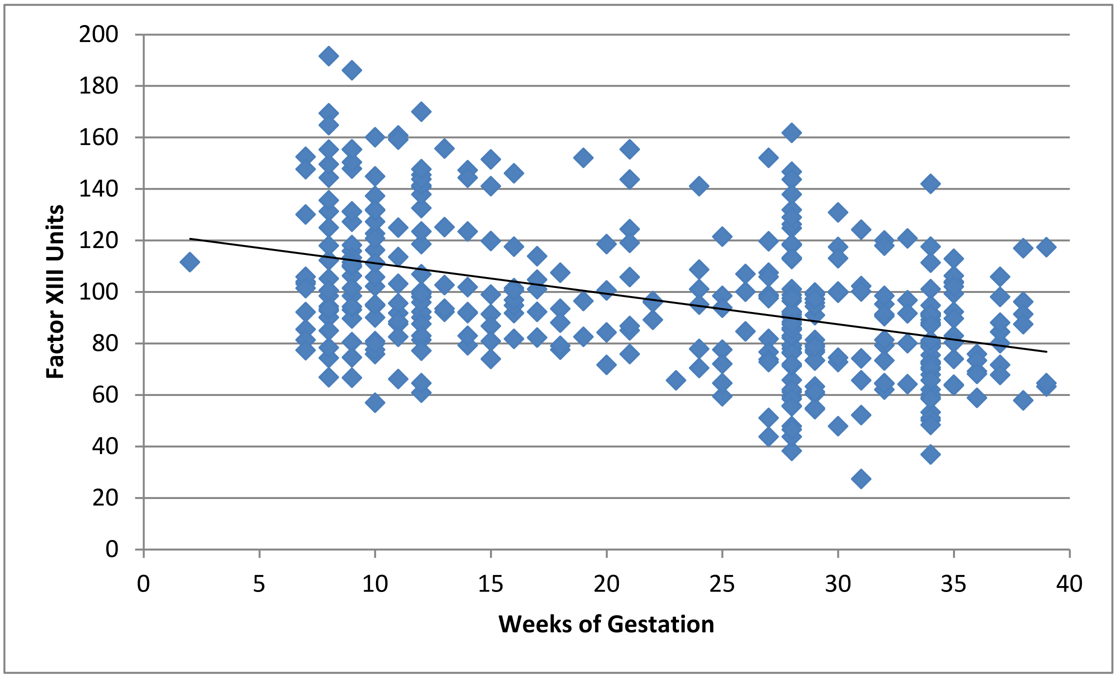

Figure 4.5 Plot and Linear regression of FXIII activity (IU/dL) in relation to the week of gestation.

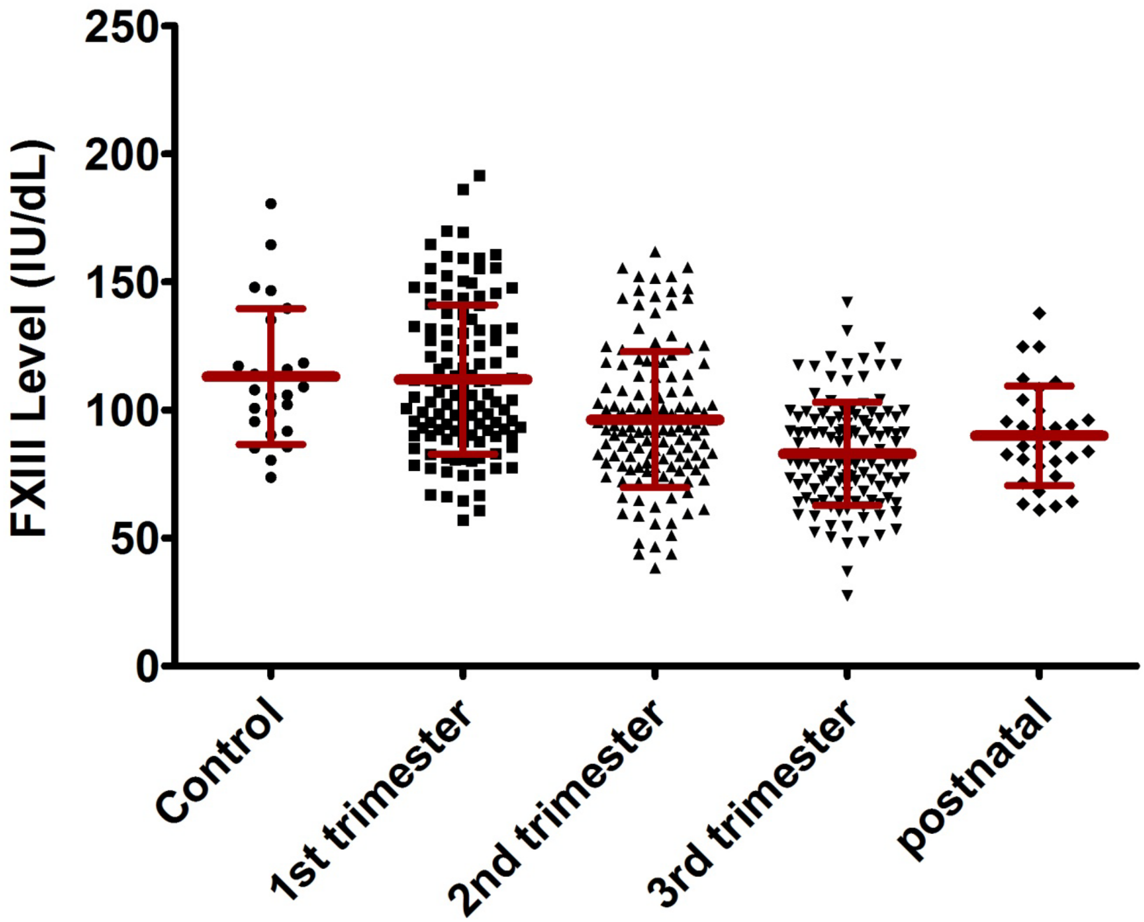

Figure 4.6 Factor XIII activity distribution among control, 1st, 2nd and 3rd trimester of pregnancy, and postnatal period. Central transverse line representing mean factor XIII activity while the upper and lower lines represent the standard deviation of the mean.

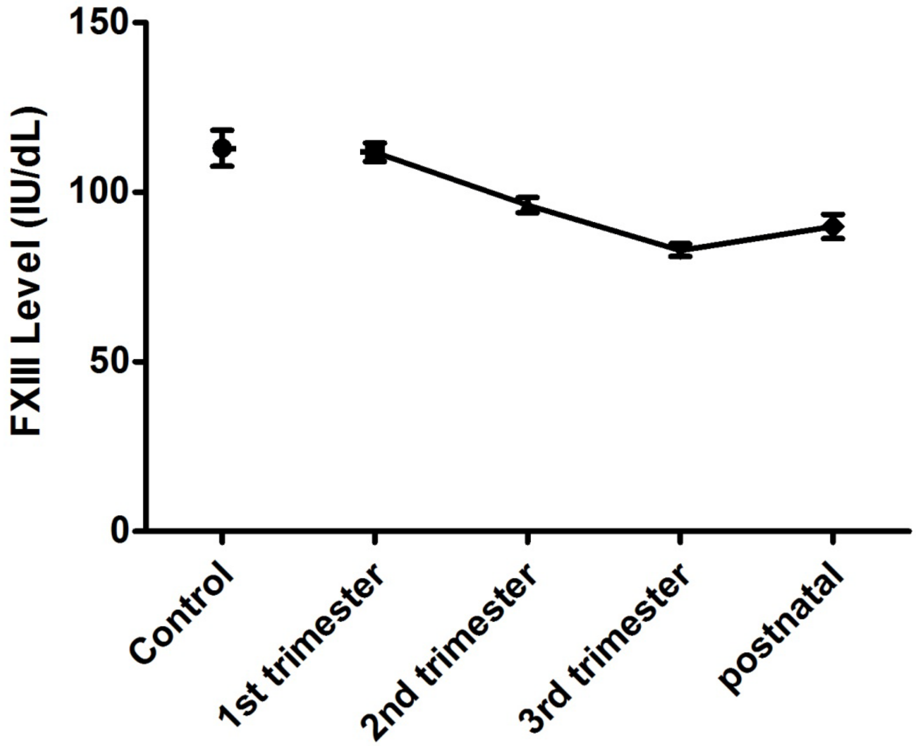

Figure 4.7 Mean and standard error of the mean for FXIII activity among control group and during each trimester of pregnancy and postnatal period.

Table 4.2 Reference intervals of FXIII activity (IU/dL) during each trimester of pregnancy and postnatal period

Reference range (centile)	**FXIII activity 1st trimester**	**FXIII activity 2nd trimester**	**FXIII activity 3rd trimester**	**FXIII activity Postnatal**
2.5%	55	45	42	61
3%	57	47	44	62
5%	64	53	49	62
10%	74	63	56	64
25%	92	78	69	77
50% (mean)	112	96	83	90
75%	131	114	98	100
90%	149	130	110	123
95%	160	139	118	131
97%	166	145	123	135
97.5%	169	147	125	137
Standard Deviation	29	26	21	19

The difference in the mean FXIII activity between each trimester of pregnancy was statistically significant with FXIII activity being lower during second and third trimester compared to the first trimester ($p<0.0001$). FXIII activity was also lower in third trimester compared to second trimester of pregnancy ($p<0.0001$). Furthermore, the mean FXIII activity during the second and third trimester of pregnancy was significantly lower compared to the non-pregnant control group (96 versus 115 IU/dL, $p = 0.001$; and 84 versus 115 IU/dL, $p <0.0001$, respectively), however, there was no significant difference between FXIII activities during first trimester compared to the control group (112 versus 115 IU/dL, $p = 0.64$).

The mean FXIII activity during postnatal period was significantly lower compared to the first ($p< 0.0001$) trimester of pregnancy but not with the second ($p= 0.2$) and third ($p = 0.12$) trimester of pregnancy. The mean postnatal FXIII activity was significantly lower when compared to control group (90 versus 115 IU/dL, $p=0.0002$).

There was a weak, positive correlation ($r=0.16$, $p=0.02$) between FXIII activity and age. FXIII activity was measured in a fresh sample, six weeks postnatally, in a cohort of ten women recruited out of 55 women with low FXIII levels in their second and third trimester of their pregnancy. In all cases, the postnatal FXIII levels had returned to the normal range.

Review of medical records for pregnancy outcome and other clinical data was available in 333 cases. Antepartum haemorrhage (APH) was noted in eight (2 %) of the pregnancies, all of these women had FXIII activity above 70 IU/dL, with one exception, who had a FXIII activity of 67 IU/dL in a sample taken during first trimester of pregnancy. The median estimated amount of blood loss post-delivery was 400 ml (range 30 to 4000 ml). Postpartum haemorrhage (PPH) was seen in 65/333 (20%) of women. Using Spearman's correlation coefficient, there was no significant relationship between FXIII activity during pregnancy and estimated blood loss post-delivery (rs= 0.56, p= 0.30).

The mean birth weight of the newborn was 3.34 kg. There were 12 (4%) infants delivered prior to 37 week of gestation, and 8 (2.4%) with low birth weight (weight <2.5 kg and born ≥ 37 weeks of gestation). There was no significant difference in FXIII activity during pregnancy in 20 women who had a low birth weight newborn (median 92, range 64-137 IU/dL) compared with those with a normal birth weight (median 91, range 27-170 IU/dL) using Mann-Whitney test (p=0.70). Similarly, there was no significant difference in FXIII activity between 20 women who delivered preterm (before 37 weeks of gestation) and 313 women delivered term babies (>37 weeks) (p=0.10).

Analysis of samples from 26 women with FXIII activity measured sequentially during at least two trimesters of their pregnancy is presented in Table 4.3. The mean FXIII activity during second and third trimester of pregnancy was lower compared to the first trimester, but the difference did not reach statistical significance and not all women had the FXIII activity measured through all three trimesters of pregnancy.

Table 4.3 Change in FXIII activity among 26 women throughout a minimum two trimesters of pregnancy.

Time point being considered		Difference in FXIII between time points			p-value*
1	**2**	**N**	**Mean difference**	**SD**	
1^{ST} trimester	2^{ND} trimester	10	-16	26	0.0836
1^{ST} trimester	3^{RD} trimester	8	-12	18	0.0947
2^{ND}trimester	3^{RD}trimester	16	-8	26	0.2340

Paired t-test; results also consistent when using non-parametric equivalent

4.4 Discussion

Reference ranges for coagulation factors during pregnancy, delivery and the immediate postpartum period have recently been established in a study including 186 women with uncomplicated pregnancies (Szecsi et al., 2010); but the FXIII was not included. This large study establishes the reference range for FXIII during the three trimesters of pregnancy and the immediate postpartum period. It confirms previous findings that FXIII significantly reduces with advancing gestation with a significant reduction in the third trimester compared to the first and second trimesters of pregnancy (Nossel et al., 1966; Coopland et al., 1969; Biland and Duckert, 1973; Hayano et al., 1982; Wersch et al., 1997).

In a previous study (Wersch et al., 1997), changes in FXIII level during pregnancy were assessed among 75 non-smoking pregnant women compared to 118 smoking pregnant women and a control group of pill-using non-smoking women. All women were ranked into four groups based on the gestational age (0-10 weeks, 11-20 weeks, 21-30 weeks, and 31-40 weeks) and FXIII level was significantly lower at 31- 40 week of pregnancy compared to 0-10 weeks of pregnancy, regardless of smoking status. This decline in FXIII level with advancing gestation is in agreement with our findings (Wersch et al., 1997).

Smoking has also been shown to affect the decline in FXIII level during pregnancy. FXIII levels during the first trimester were significantly higher in smokers compared to the control group, but it was not significant in the non-smokers. In addition, a decline of XIII level started from 20 weeks of gestation for non-smoking women, but from 30 weeks of gestation for smokers (Wersch et al., 1997), and smokers had a significantly higher level of FXIII compared to non-smokers during the second half of pregnancy. It has been speculated that the rise of FXIII is due to relative polycythaemia and platelet activation. In our study there were only seven smokers, an insufficient number to establish any possible correlation with smoking.

In a further study of 39 pregnant women and a control group of 10 non-pregnant women, a steady decline in plasma FXIII concentration was reported with advancing gestation. The study showed no significant difference between the samples taken 12-20 weeks of gestation compared to the control group. However, the mean FXIII level was significantly lower during 20-30 weeks of pregnancy and 30 weeks to term compared to the control group. The method of analysing FXIII was the clot solubility assay; this incorporates ^{125}Iodine-labelled fibrinogen, to provide a quantitative endpoint by measuring the radioactivity of clot supernatant (Coopland et al., 1969).

In our study, ten of the women with FXIII activity <70 IU/dL were found to have normal FXIII levels when they were retested during the immediate postpartum period. It is not clear whether the reduction of plasma FXIII during pregnancy represents reduced synthesis of FXIII-A, increased consumption, or is due to haemodilution. In normal pregnancy, there is an average 50% increase in maternal blood plasma volume, beginning around the 10th week of gestation up to the 34-36th week of gestation (Hytten, 1985). However, other coagulation factors such as factor VII, VIII, and IX tend to increase during pregnancy despite hemodilution (Bremme, 2003), possibly due to an increase in the synthesis of these factors. In a study of coagulation factors among newborn and their mothers, the FXIII activity in 50 women was measured shortly before delivery and found to be lower than the FXIII level in the cord blood of their babies. The authors speculated on a possible passage of FXIII to the fetal circulation to explain the reduction in maternal plasma FXIII level near delivery (Biland and Duckert, 1973).

Another study compared FXIII changes in the third trimester of normal pregnancy and pregnancies complicated by intrauterine growth retardation (IUGR). FXIII level was maintained in the IUGR group, while in the normal pregnancy, there was a significant reduction compared to mid-pregnancy (Persson et al., 1980). This suggests that the reduction in FXIII level seen during the third trimester of pregnancy is related to maternal FXIII transfusion through the placenta to the fetus. Those with IUGR fail to consume maternal FXIII possibly due to reduced placental transfusion to the fetus, thus maintaining higher plasma FXIII during the third trimester of pregnancy. A further possible explanation is that placental growth and expansion requires an increasing consumption of maternal FXIII due to its role in trophoblastic invasion, resulting in a progressive reduction of plasma FXIII level toward the end of pregnancy. IUGR is associated with poor growth and development of the placenta and thus fails to consume maternal FXIII.

In conclusion, our study demonstrates a progressive decline in FXIII levels during the three trimesters of pregnancy and provides the reference range for FXIII level during pregnancy. It also shows a reduced FXIII levels in the immediate postnatal period. Knowledge of the FXIII reference range is helpful for appropriate management of FXIII deficiency in pregnancy. It can also improve our understanding of the role of FXIII in pregnancy related complications. Further studies are required to assess the role of FXIII level and its changes in pregnancy complicated by placental insufficiency and IUGR.

CHAPTER FIVE

PLASMA FXIII LEVEL VARIATIONS DURING MENSTRUAL CYCLE

5 CHAPTER FIVE: PLASMA FXIII LEVEL VARIATIONS DURING MENSTRUAL CYCLE

5.1 Introduction

Many physiological variables affect coagulation factor levels, including age, sex, race, blood group and female sex hormones. Cyclical variations of the female sex hormones have been reported during the menstrual cycle (Kadir et al., 1999a; Knol et al., 2012).

During the menstrual phase of the cycle, FXIII could be important in the process of healing the endometrium and controlling the menstrual blood loss through fibrin cross-linking, thus improving the mechanical strength, rigidity and elasticity of the clot and prevent it from being dissolved through fibrinolysis (Muszbek et al., 1996). However, the effect of the menstrual cycle on FXIII levels is not been fully understood and it is also not clear whether endometrial healing leads to an increase in FXIII consumption, thus altering the plasma level. Variations in FXIII level during the menstrual cycle, if present, would need to be considered when performing screening tests for FXIII deficiency. The aim of this study was to examine possible changes in plasma FXIII activity during the normal menstrual cycle and to assess any correlation between FXIII activity during the menstrual phase and the menstrual blood loss.

5.2 Materials and Methods

5.2.1 Study design:

This was a longitudinal study of 32 women of reproductive age. Women were recruited from hospital staff through leaflet and poster invitations at Royal Free Hospital NHS Foundation Trust, from January to October 2012. Women were included in the study if they had a regular menstrual cycle of 26-35 days duration, and were not using hormonal contraception, including *Mirena*® IUS (levonorgestrel-releasing intrauterine system), and were not using non-steroidal anti-inflammatory drugs. Exclusion criteria also included personal or family history of bleeding tendency or thrombo-embolism. The study was approved by the hospital ethical committee and informed consent was obtained from participating women.

Demographic and clinical data collected include: age, ethnicity, previous number of pregnancies and miscarriages, body mass index (BMI), and smoking status through an interview with the women.

Menstrual blood loss was measured using the pictorial blood-assessment chart (PBAC) (Higham et al., 1990). The women were given oral and written instructions on the use of PBAC and were asked to complete the chart during the period of blood sample collection. Women were required to document the number of sanitary pads and/or tampons used each day (24 hour) based on the degree of saturation of the pads/tampons, they were also asked to record the number and size of blood clots if present (Appendix 4). The completed PBAC sheets were collected at the end of the menstrual period and the total numerical score were calculated by Dr. Lava Sharief. The total score was calculated by adding up the pad/tampons counts obtained from each day of the period. The scores assigned for tampons were "1" for each lightly stained tampon, "5" if moderately stained and "10" if it was completely saturated with blood. The towels were given ascending scores of "1", "5" and "20". Small and large clots scored "1" and "5", respectively PBAC score $\geq$ 100 were considered a heavy menstrual bleeding (Higham et al., 1990).

A bleeding questionnaire was also completed and the bleeding score was calculated based on specific symptoms; these included epistaxis, cutaneous symptoms, bleeding from minor wounds, muscle hematomas, haemarthrosis, oral cavity bleeding, gastrointestinal bleeding, bleeding after tooth extraction, bleeding after surgery, postpartum haemorrhage, and menorrhagia. The severity of each symptom was subsequently summarised, using a bleeding questionare system ranging from "-1" representing absence of bleeding despite haemostatic challenge, "0" representing complete absence of bleeding symptoms, "1" given when patient reported presence of bleeding, "2" if the bleeding symptoms required evaluation by a physician but no active intervention was needed, "3" if bleeding required some kind of intervention by physician, and "4" if blood transfusion or surgery was required to control the bleeding (Tosetto et al., 2007). Doctor Lava Sharief was trained to complete the questionnaire and score the sheet by a haematologist in the department (Appendix 5).

5.2.2 Laboratory method:

Blood samples were taken four to six times during one month period as follows: menstrual phase (day 1-5), proliferative phase (day 6-11), periovulatory phase (day 12-17), secretory phase (days 18-23), and premenstrual phase (day 24-29).

After obtaining written informed consent, blood samples were obtained between 11:00 a.m. and 2:00 p.m. after a resting period of 10 minutes. Venous blood samples were collected by clean venepuncture, with minimal stasis, into citrate (0.105mol/L) Vacutainers™ (BD Diagnostics, Oxford, UK), with a ratio of one part anticoagulant to 9 parts whole blood. Within one hour of collection platelet poor plasma (PPP) was prepared by double centrifugation at ambient temperature (2000g for 10 minutes) and aliquots of PPP were frozen to -80ºC. On the day of assay samples were thawed to 37ºC.

The laboratory method to measure plasma FXIII level was the chromogenic ammonia release assay as described in chapter 4.

5.2.3 Statistical method:

This study is a pilot study. Practical and statistical constraints suggested that 30 women was a feasible recruitment number. Current practice suggests that the normal range for Factor XIII activity in women is 70 to 140 IU/dL. Thus, we assumed a mean level of 105 IU/dL with a standard deviation of 20 IU/dL between women. A single previous study of eight women (Bolis et al., 1982) has investigated the within-woman variation in FXIII levels during the menstrual cycle, and found a change of around 30% from late follicular phase compared to levels during menstruation (i.e. levels at late follicular phase were 67.2% of those seen during menstruation).

Taking this information, we assumed that the mean change in FXIII will be a reduction from 105 to 71 IU/dL. However, we assumed a more conservative drop of 15 IU/dL per woman between the two time points, and a conservative within-woman SD of 24 IU/dL. Assuming the primary analysis is performed by comparing changes in FXIII between these two time points using a paired t-test and a Type I error of 5%, this sample size of 30 women gives a power of 80%.

The statistical package for social sciences, version 20 (SPSS, Chicago, USA) was used. Continuous data were presented as mean ± standard deviation (SD). Paired sample t-test was used to compare FXIII activity in different phases of the menstrual cycle to the menstrual phase. Comparison of FXIII changes in relation to PBAC score and body mass index (BMI) was performed using an unpaired t-test ($p \leq 0.05$ was considered statistically significant). Because statistical test was performed between four groups, Bonferroni correction was performed in order to and p value was considered significant if <0.0125

5.3 Results

The demographics of 32 women included in this study are illustrated in Table 5.1. All women included in the study were found to be non smokers. Only eight women had a previous history of pregnancy with a total of 20 pregnancies; four of them resulted in miscarriage. The median PBAC score was 92, ranging from 12 to 920. Bleeding scores were “-1” in seven (22%) women, “0” in 22 (68%) women, “1” in one (4%), and “3” in two (6%) women. In total, 153 blood samples were collected from the women.

Table 5.2 shows the mean FXIII level during various phases of the menstrual cycle while Figure 5.1 and Figure 5.2 show the distribution and the mean trend of FXIII activity during each phase of the menstrual cycle. The mean FXIII level was lowest during the menstrual and periovulatory phases of the cycle (114 IU/dL) and highest level in the secretory (121

IU/dL) and premenstrual (122 IU/dL) phases and this difference was found to be statistically significant (p=0.036).

Table 5.1 Demographics of 32 women with normal menstrual cycle included for this study

Variables	**Outcome**
Country of origin, No. (%)	
Afro-Caribbean	6 (19)
UK	9 (28)
Asian	7 (22)
Germany	5(16)
Poland	1(3)
Iranian	1 (3)
Spanish	1 (3)
Turkey	2 (6)
Women with previous miscarriage No. (%)	
Yes	4 (12,5)
No	28 (87.5)
Previous pregnancies No. (%)	
None	24 (76)
1	2 (6)
2	2 (6)
3	2 (6)
4	2 (6)
BMI (Kg/m^2); Mean ±SD (range)	25± 4.5 (19 to 36.5)
Age (years); Mean ±SD (range)	28.8± 8 (18 to 42)
Height (cm); Mean ±SD	162 ±7
Weight (kg); Mean ±SD	66 ±14

Table 5.2 FXIII level (IU/dL) at different phases of menstrual cycle in 32 healthy women

	Week 0 (Menstrual)	**Week 1 (proliferative)**	**Week 2 (periovulatory)**	**Week 3 (Secretory)**	**Week 4 (premenstrual)**
Number samples	32	32	32	31	22
Mean	114	119	114	121	122
SD	23	26	21	22	27
Lower range	68	69	72	78	68
Upper range	248	254	255	273	256
p-value*	-	0.058	0.997	0.036	0.036

* paired t-test.

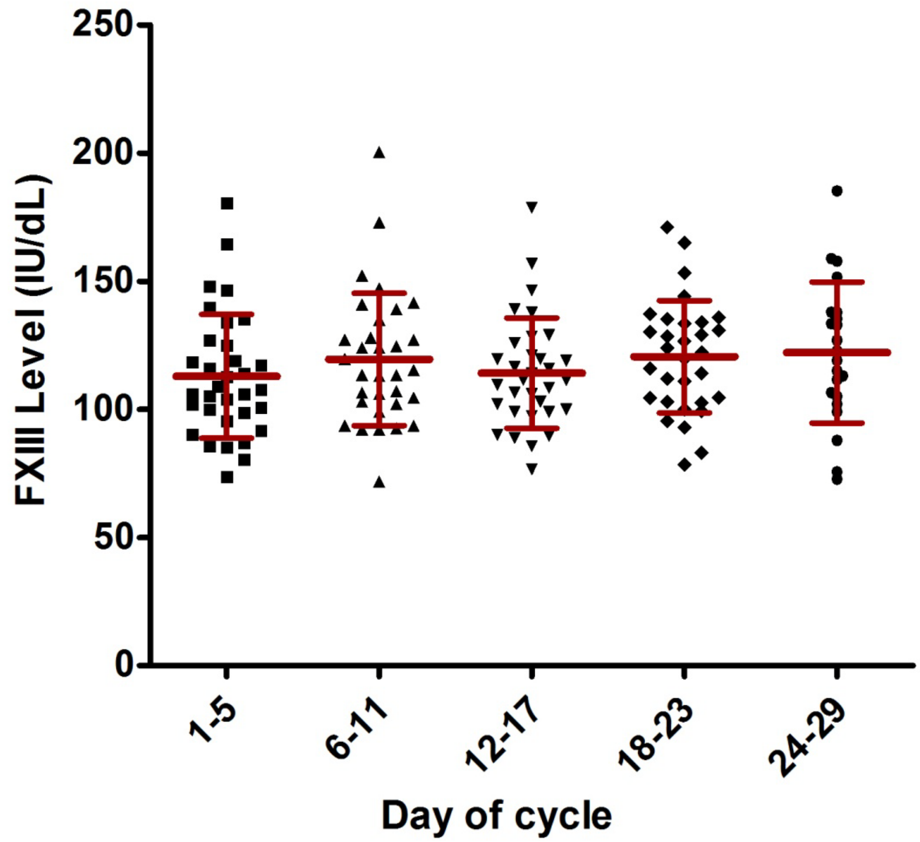

Figure 5.1 Distribution of FXIII activity (mean ± SD) during each phase of the menstrual cycle

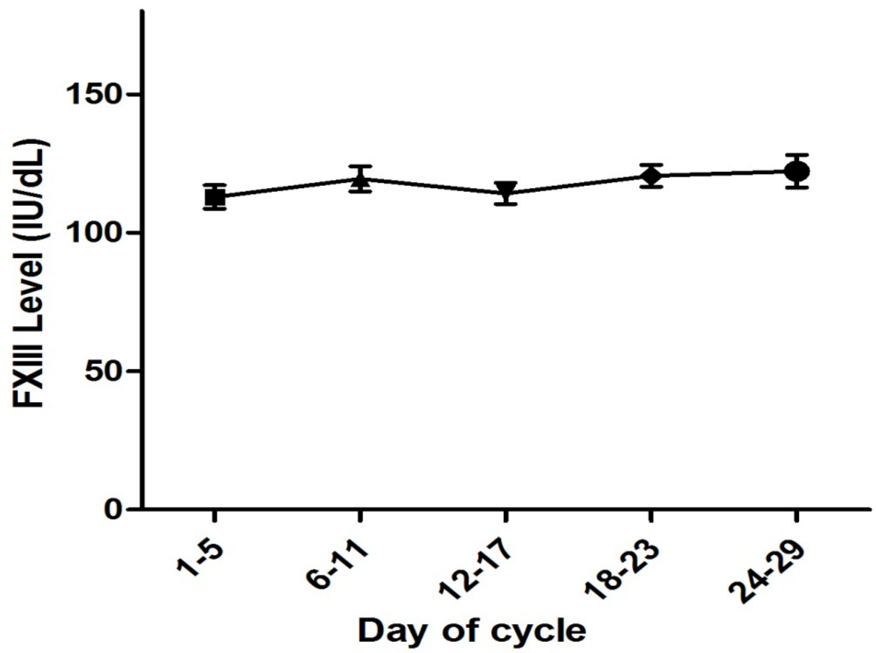

Figure 5.2 Mean and standard error of the mean for FXIII activity and its trend throughout menstrual cycle

There was no significant correlation between FXIII activity during the menstrual phase and age ($p= 0.53$) or PBAC score ($p=0.53$), using Spearman's correlation coefficient. Among the 14 women with PBAC score $\geq$ 100, the median FXIII activity during the menstrual phase of the cycle was 116 IU/dL, this was not statistically different from women with a normal PBAC score (113 IU/dL) with p 0.72 (unpaired t-test). There was no correlation between FXIII and bleeding scores.

The women were divided into those with BMI less than 25 kg/m2 (n=13) and greater than 25 kg/m^2 (n=11). No significant difference was observed in FXIII activity at different phases of menstrual cycle between the two groups (Table 5.3).

Table 5.3 FXIII activity (Mean and SD) at different phases of the menstrual cycle between normal and overweight women

Menstrual Cycle phase	**FXIII at BMI <25 kg/m2 (N=13)**	**FXIII at BMI >25 kg/m2 (N=11)**	**p value***
Day 1-5 (Menstrual)	111 (25.1)	119 (27.0)	0.46
Day 6-11 (Proliferative)	116(31.6)	125 (26.5)	0.48
Day 12-17 (Periovulatory)	112(25.6)	121 (20.9)	0.37
Day 18-23 (Secretory)	120 (22.9)	122 (21.6)	0.87
Day 24-29 (Premenstrual)	130 (35.0)	120 (32.4)	0.59

Variables presented as mean (SD), * Tested using unpaired t-test

5.4 Discussion

In this study, FXIII activity was lowest during the menstrual and periovulatory phases. The level of FXIII was significantly higher toward the last two weeks of the cycle compared to the menstrual phase. FXIII activity during the menstrual cycle has been studied in one longitudinal study by Bolis *et al*. In that study, blood samples were taken from ten healthy women on four occasions during the menstrual cycle. The authors showed a highly significant decrease in mean FXIII level during the peri-ovulatory phase (67 IU/dL) compared to secretory (85 IU/dL) and premenstrual phases (89 IU/dL) with $p<0.001$ (Bolis et al., 1982). However, the FXIII level was not measured during the first five days of the cycle (menstrual phase).

Our study has demonstrated that the FXIII levels can be influenced by natural hormonal changes during the menstrual cycle. The lower plasma FXIII activity during menstrual and periovulatory phase compared to other phases of the cycle might be attributed to the increase in the consumption of this factor for its role in healing, angiogenesis and fibrin stabilisation needed during these phases of the cycle. FXIIIa induces a severe reduction in the thrombospondin-1 (TSP-1) mRNA and reduce TSP-1 levels in the conditioned medium. TSP-1 acts as an angiogenesis inhibitor by preventing endothelial cell proliferation and migration, as well as inducing apoptosis (Lawler, 2002). Further mediators for FXIII pro-angiogenic effects involve upregulation of certain transcription factors, and tyrosine phosphorylation and activation of vascular endothelial growth factor receptor-2 (Dardik et al., 2005; Muszbek et al., 2011b). This proangiogenic function of FXIII might be important in endometrial healing, regeneration and vascular reproliferation following endometrial layer shedding during menstrual cycle as well as ovarian surface at site of follicular rupture during ovulation.

This study did not find any correlation between FXIII level during menstrual phase and menstrual bleeding. There was no significant difference in FXIII activity in women with heavy menstrual bleeding (PBAC score equal or more than 100) compare to those with normal menstrual bleeding. However, it is important to note that the PBAC was used in this study to measure menstrual blood loss and this is a simple tool that only allows a semi objective assessment of menorrhagia (Kadir et al., 1999b). The PBAC was chosen rather than a more objective assessment tool, like alkaline hematin, because it is more practical and less expensive.

Understanding these changes in FXIII during menstrual cycle will help clinicians to perform blood tests during appropriate phase of the menstrual cycle and to capture the baseline level. The sensitivity of testing for FXIII deficiency can also be increased by avoiding testing during menstrual and peri-ovulatory phases of the cycle, which are associated with a low FXIII level. This is of paramount importance in the diagnosis and management of women with bleeding disorders. For those affected with mild FXIII deficiency, knowledge of FXIII changes during menstrual cycle can help the clinician time surgical interventions during the phase with the highest coagulation factor level. This may help reduce the risk of bleeding and reduce the need/ the amount of haemostatic cover and this in turn, reduces the risk of exposure to unnecessary plasma products.

CHAPTER SIX

FACTOR XIII LEVEL IN WOMEN WITH RECURRENT MISCARRIAGES

6 CHAPTER SIX: FACTOR XIII LEVEL IN WOMEN WITH RECURRENT MISCARRIAGES

6.1 Introduction

Recurrent miscarriage, defined as spontaneous loss of three or more consecutive pregnancies occurring before week 20 of pregnancy, affects 1% of women of reproductive age (Rai and Regan, 2006). Although many of the cases are related to identifiable maternal or fetal abnormalities, the cause of most cases of recurrent miscarriage remains unknown (Li et al., 2002). An adequate utero-placental circulation is essential for maintaining pregnancy; this is achieved by sustaining a stable maternal coagulation and fibrinolytic system (Buchholz and Thaler, 2003).

Women with severe congenital FXIII deficiency are a risk factor of recurrent early pregnancy loss (Rodeghiero et al., 1987; Inbal and Kenet, 2003; Asahina et al., 2007). Systemic review of literature in chapter 3 showed an overall miscarriage rate of 66% among women with severe congenital FXIII deficiency. The miscarriage rate was mainly in those not receiving FXIII prophylactic treatment, with a rate of 91% (please refer to the results in chapter 3). In a study on 351 women with recurrent miscarriage, seven (2%) of the women had a bleeding disorder, one of whom had FXIII deficiency (Bick and Hoppensteadt, 2005). The minimum FXIII level of 31 IU/dL has been suggested to be necessary for maintaining pregnancy in a patient with FXIII deficiency (Peyvandi et al., 2012). However, it is not known whether a borderline FXIII level (levels below 70 IU/dL) is associated with an increased risk of recurrent miscarriage (Inbal and Muszbek, 2003) and whether FXIII assessment should be part of the investigation for women with miscarriage. The aim of this study is to examine the plasma FXIII level in women with a history of recurrent miscarriage compared to a control group of women with no miscarriage and one living child to establish any association between FXIII level and recurrent miscarriage.

6.2 Materials and methods

6.2.1 Study design

This case-control study included 68 women (30 women were recruited at The Royal Free Hospital NHS Foundation Trust and 38 women were recruited at Kings College Hospital NHS Foundation Trust). The study was approved by the hospitals ethical committees and informed consent was obtained from participating women.

The women included were cases with a history of recurrent miscarriage referred for evaluation to the recurrent miscarriage clinics by their general practitioner. The European Society for Human Reproduction and Embryology (ESHRE), defines recurrent miscarriage as three consecutive pregnancy losses occurring before 20 weeks of gestation (Jauniaux et al., 2006). All women underwent a detailed evaluation to exclude other possible causes for their miscarriages. Clinical assessment included physical examination, vaginal ultrasound examination, thrombophilia screen, hormonal levels (FSH, LH, Progesterone, Prolactine), lupus anticoagulant testing, tests for anti-phospholipid antibodies (IgG and IgM anti-cardiolipin antibodies), thyroid hormone function tests, and chromosomal analysis for both partners. Women were included in the study when the results of all previous investigations were normal and women attending the clinic for follow-up visit. During this stage, blood samples were taken at least two months following the last pregnancy loss for the purpose of measuring FXIII level.

The control group consisted of 62, age matched, healthy women (30 women recruited from The Royal Free Hospital NHS Foundation Trust, 32 women were recruited from Kings College Hospital NHS Foundation Trust) from hospital staff recruited for this study through poster and leaflet invitations. All control women had no history of miscarriages and at least one successful pregnancy with a live child. Women were excluded if there was history of thrombo-embolic events or bleeding disorders and those on hormonal contraceptives at time of blood sampling. Other exclusion criteria included those on aspirin and non steroidal anti-inflammatory medications within five days prior to blood sampling.

6.2.2 Laboratory Methods:

Blood samples were obtained between 1:30 p.m. and 4:00 p.m. after a resting period of 10 minutes. Venous blood samples were collected by clean venepuncture, with minimal stasis, into citrate (0.105 mol/L) Vacutainers™ (BD Diagnostics, Oxford, UK), with a ratio of one part anticoagulant to 9 parts whole blood. Within one hour of collection platelet poor plasma (PPP) was prepared by double centrifugation at ambient temperature (2000g for 10 minutes) and aliquots of PPP were frozen to -80ºC. On the day of assay samples were thawed to 37ºC.

The method of measuring plasma FXIII level is the same described in the material and method section of chapter 4.

6.2.3 Statistical Analysis:

The sample size for this study was chosen based on a power calculation that suggested 60 women would be adequate recruitment number. Current practice suggests that the normal range for Factor XIII activity in women is 70 to 140 IU/dL. Thus, we assumed a mean level of 105 IU/dL with a SD of 20 IU/dL between women. In a previous study of 26 women (Schubring et al., 1990) who presented with spontaneous miscarriage, FXIII activity was significantly lower by 15 % compared with a control group of normal pregnant women during the same gestational period. Taking this information, we assumed that the mean change in FXIII will be a difference from 105 to 98 IU/dL. However we assumed a more conservative drop of 15 IU/dL per woman between the two groups, and assumed a conservative, within-woman, SD of 24 IU/dL. Assuming the primary analysis is performed by comparing changes in Factor XIII between these two groups using a non-paired t-test and a Type I error of 5%, this sample size of 60 women gives a power of 80%.

Numerical variables were presented as mean and their standard error (SE). Comparison of the mean FXIII levels between cases and controls was performed using unpaired sample t-test. For categorical variables, a chi-square test was performed, adjusted with Fisher exact test when necessary. Statistical analyses utilised SPSS®, version 20 (SPSS, Chicago, USA). The level of statistical significance was set at $p < 0.05$.

6.3 Results:

Among women with recurrent miscarriage, the median number of miscarriages was three (range 3-6). The mean age ± SE was 35 ±0.7 years and 33 ±1.0 years in women with recurrent miscarriages and control group, respectively with no significant difference between both groups (p=0.142). The median gestational age at time of the first, second, and third miscarriage was 8.0 (range 2-18), 8.0 (range 4-17), and 7.0 (range 4-19) weeks, respectively.

As seen in Figure 6.1, the mean ±SE FXIII activity among women with recurrent miscarriage was 105±4.0, range 40-205. In the control group, mean ±SE was 111±2.9, range 48-181 IU/dL. There was no statistical significant difference between these two levels (p=0.23). Eight (12%) women with recurrent miscarriage had FXIII activity < 70 IU/dL; this was significantly higher compared to control group with only one (2%) woman having FXIII activity <70 IU/dL (p= 0.034, Fisher exact test). Among the cases with recurrent miscarriage, 39 (57%) women had primary miscarriages (defined as recurrent miscarriage with no prior live birth) with mean ± SE FXIII activity of 106 ±5.5 IU/dL, while 29 (43%) women with a history of secondary miscarriage (defined as recurrent miscarriage after a prior live birth) showed a FXIII level of 103 ±6.0 IU/dL. This difference was also not statistically significant (p=0.75).

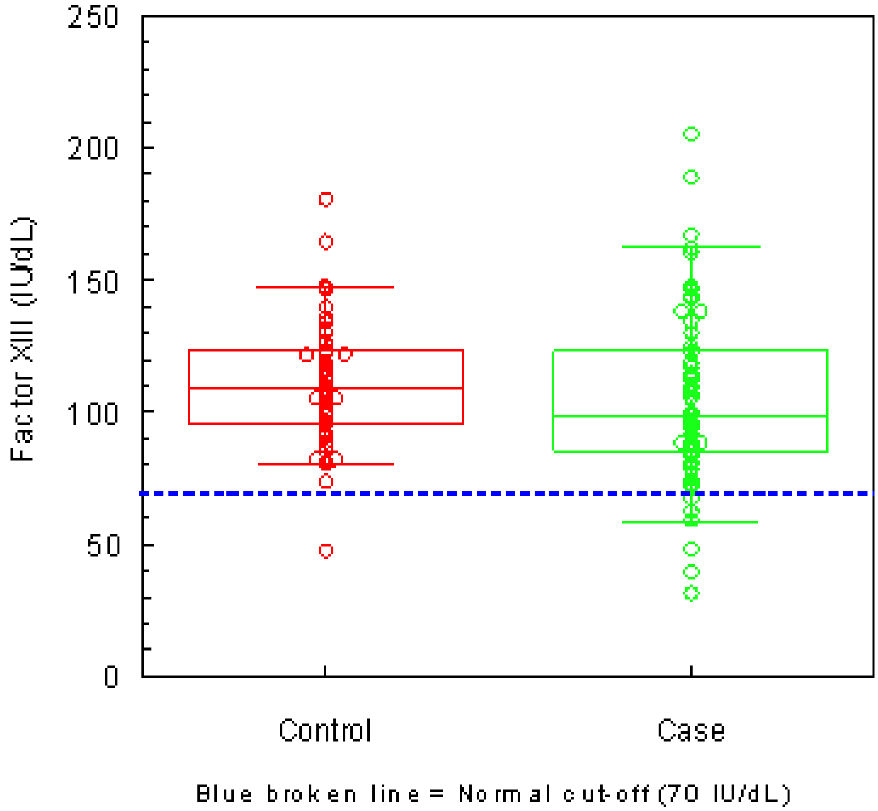

Figure 6.1 FXIII activity mean and distribution in women with history of recurrent miscarriage compare to control group

6.4 Discussion

This study aimed to investigate the presence of any possible link between level of plasma FXIII and occurrence of unexplained recurrent miscarriage in otherwise healthy women. This study did not show a statistically significant difference in the mean FXIII level in women with history of recurrent miscarriage compared to a control group; however, there was a significant difference in the distribution of FXIII level with significant higher number of women with low FXIII activity < 70 IU/dL in the recurrent miscarriage group compared to the control group.

Previous studies have tried to examine the association between plasma FXIII level and recurrent miscarriage (Ogasawara et al., 2001; Pasquier et al., 2012). In a study among 424 women with history of recurrent miscarriage, FXIII activity was measured at week four of pregnancy. The women were followed up prospectively for the occurrence of miscarriage. The study showed no significant difference in the miscarriage rate between those with normal versus abnormal FXIII level [47/186 (25.3%) versus 2/10 (20.0%)]. Their normal range of FXIII was 52–118 IU/dL, determined by measuring 50 healthy individuals (Ogasawara et al., 2001). This study did not compare absolute plasma FXIII activity between women with and without consecutive miscarriages, and there was no similar comparison with a control group. Furthermore, the definition of the normal range applied in this study is debatable as it was based on a small number of individuals.

In a further study of 26 women presented with spontaneous miscarriage (between 6-14 weeks of gestation), FXIII activity was significantly lower by 15 % compared with a control group of normal pregnant women during same gestational period. The FXIII activity in the group of women with miscarriage returned back to normal when examined six weeks after the pregnancy loss (Schubring et al., 1990). One limitation of the study is the lack of comparison with a control group of non pregnant women. In addition, it is difficult to assess whether the lower level of FXIII seen in the miscarriage group is the cause or the result of the miscarriage process.

A more recent study assessed FXIII-A and FXIII B-subunit antigen levels in 264 women with two or more miscarriages in comparison to a same number of control group (women with no history of miscarriage and had one living child) and found no significant difference between both groups. However, analysis of a subgroup of 152 women with three or more miscarriages (early miscarriage before 20 weeks) showed a slightly higher FXIII-A (100.8% vs. 96.0%, P = 0.027, OR 1.011) and FXIII-B (97.6% vs. 93.2%, P = 0.023, OR 1.012) antigen levels in the women with recurrent miscarriage compared to controls. The study showed no association between FXIII-A and B antigen levels and pregnancy loss, except for a reduced odd ratio for pregnancy loss below the 75^{th} (108 IU/dL) and 50^{th} (90 IU/dL) percentiles, respectively, and only for a subgroup of women with three or more recurrent miscarriage. No similar association with FXIII antigen level below 10^{th} percentile (70 IU/dL) and 25^{th} percentile was seen (Pasquier et al., 2012). The study, however, did not assess FXIII activity. There were 24 women with FXIII-A antigen levels of < 70 IU/dL in the cases compared to 22 women in the control group. The authors thus suggested that the mild FXIII-A deficiency seen in the patients and controls is not related to pregnancy loss (Pasquier et al., 2012).

Successful implantation involves adequate cytotrophoblast growth to the proper depth of the endometrium, thus providing adequate anchorage for the conceptus and promotes adaption of uteroplacental circulation (Feng et al., 2000). The formation of the placental basal plate matrix, known as Nitabuch layer, involves the activation of the intravenous maternal procoagulant cascade, leading to fibrin deposition on the walls of decidual veins around the areas of trophoblastic invasion (Craven et al., 2002). FXIII plays an important role through forming γ-glutamyl-ε-lysine peptide covalent bonds that cross-links fibrin γ- and α-chains (Ariëns et al., 2000). Thus, FXIII behaves as an adhesive protein, promoting the binding of fibronectin receptors between the stroma and cytotrophoblasts.

FXIII also adheres to fibrinogen and type IV collagen located at the surface of decidual cells; thus strengthening the attachment of cytotrophoblast in the endometrium. However, in the absence of FXIII, fibrin polymers are aggregated only by weak intermolecular hydrogen bonds (Asahina et al., 1998). Immuno-histochemical staining of implantation tissues in women with congenital FXIII deficiency showed poorly formed cytotrophoblastic shells and Nitabuch's layers together with undetectable FXIII at placental bed compared with implantation tissues of healthy women (Asahina et al., 2000).

Possible mechanisms for first trimester pregnancy loss in women with FXIII deficiency include a coagulation defect leading to micro-vascular bleeding resulting in fetal loss (Hsieh and Nugent, 2008). However, it is not clear whether impaired coagulation alone is the primary cause of miscarriage considering the multifunctional nature of FXIII. Another possible hypothesis is the influence of FXIII on the innate immune system. FXIII affects the complement immune system through incorporating C3 in the fibrin clot and prolonging fibrinolysis (Wang et al., 2010; Bagoly et al., 2012). It also augments monocyte and macrophage phagocytic activity and enhances platelet binding to monocyte, which in turn localise immune response to the area of injury (Ichinose, 2012). Whether other links exist between FXIII and the immune system as a cause of recurrent miscarriage is not fully known and would require further studies in the future.

Although the mean difference in FXIII activity was not statistically significant among women with recurrent miscarriage compared to the control group, there were a significantly higher number of women with recurrent miscarriage who had FXIII activity < 70 IU/dL compared to the control group. It is not clear whether the low level of FXIII activity in this subgroup of women is linked to their risk of recurrent miscarriage. FXIII may cross-link substrates other than fibrin that are also important for maintaining pregnancy. Some heterozygous mutations alter the structure of FXIII with no direct effect on FXIII level and FXIII activity. Whether altered FXIII structure contributes to the risk of miscarriage is also a possibility. In addition, the presence of other factors (haemostatic, immunological, others) may have a synergistic effect on the role of FXIII in inducing pregnancy loss. A study on artery thrombotic tissues and pulmonary emboli concluded that PAI-1 polymorphism, in combination with FXIII Val34Leu polymorphism have synergestic effect and can increase the risk of fibrinolysis impairment through inhibiting the fibrinolytic system activity and increasing fibrin network resistance against fibrinolysis (Kohler, 2001). However, this synergistic effect was not found in a more recent study comparing 63 women with recurrent miscarriage to a control group and found only PAI-1- 4G polymorphism to be a risk factor for pregnancy loss but no similar role of FXIII Val34Leu polymorphism (Abalovich et al., 2002).

In conclusion, this study is the first study to assess FXIII activity in women with history of recurrent miscarriage and control group of women with no miscarriage and one living child. It showed that significantly higher number of women with recurrent miscarriage have FXIII level < 70 IU/dL. It is possible that borderline FXIII activity has a role in pregnancy loss in this subgroup of women. Advocating for FXIII level screening among women with recurrent miscarriage would require further studies in order to assess their overall systemic and local haemostatic parameters.

CHAPTER SEVEN

OVERALL CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

7 CHAPTER SEVEN: OVERALL CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

7.1 Overall conclusions

In conclusion, the systematic review of 192 pregnancies in 121 women with congenital FXIII deficiency revealed heavy menstrual bleeding to be a common gynaecological problem with a prevalence of 26%. However, this is most likely to be an underestimate as most case reports did not provide information on menstrual bleeding. Ovulation bleeding was also common and observed in 8% of affected women. Pregnancies in women with FXIII deficiency have a significant risk of miscarriage, placental abruption, and PPH if not treated. The prevalence of miscarriage in women not on prophylaxis reached as high as 91%. Therefore, replacement therapy is essential to achieve successful pregnancy and to reduce the risk of bleeding during pregnancy, preterm delivery and PPH.

Our longitudinal study of 32 women of reproductive age during the normal menstrual cycle found the mean FXIII level to be lowest during the menstrual and periovulatory phases of the cycle (114 IU/dL) and highest in the secretory (121 IU/dL) and premenstrual (122 IU/dL) phases and this difference was found to be statistically significant ($p=0.036$). There was no significant correlation during the menstrual phase, between FXIII activity and menstrual blood loss assessment score ($p=0.53$).

The cross sectional study on 376 women during pregnancy and immediate postpartum period demonstrated a significant difference in the mean FXIII activity of 112 IU/dL during the first trimester compare to second (96 IU/dL) and third (83 IU/dL) trimester as well as postnatal period (90 IU/dL). The reference range for FXIII activity during the first trimester was 55 - 169 IU/dL, for the second trimester was 45 -147 IU/dL, for the third trimester 42 - 125 IU/dL and for postnatal period was 61 -137 IU/dL. Knowledge of the FXIII reference range is helpful for appropriate management of FXIII deficiency in pregnancy. It can also improve our understanding of the role of FXIII in pregnancy complications.

Our case-control study included 68 women with recurrent miscarriage compared with a control group of 62 women without history of miscarriage and with at least one successful pregnancy. The mean FXIII activity among women with recurrent miscarriage was 105 IU/dL, while the control group had a mean FXIII activity of 111 IU/dL. There was no statistical significant difference between these two levels (p=0.23). Eight (11.8%) women with recurrent miscarriage had FXIII activity < 70 IU/dL; this was significantly higher compared to control group with only one (1.6%) woman having FXIII activity <70 IU/dL.

This thesis highlighted the significant obstetrics and gynaecological morbidity in women with congenital FXIII deficiency. The thesis also showed that FXIII level changes significantly under hormonal influence during menstrual cycle and during pregnancy. The reference range for FXIII level is established during pregnancy and immediate postpartum period. The role of FXIII in women with recurrent miscarriage is also explored and highlighted the need for further studies.

7.2 Suggestions for future research

7.2.1 Scope for future guideline

Due to the rarity nature of FXIII deficiency, multicenter studies and/or a web based international registry are necessary to properly examine the pregnancy and delivery management and outcome in these women. This will also create more evidence to base the guidelines for management upon, to ensure best outcome for the mother and her expected neoborn.

Increased awareness among clinicians and a multidisciplinary approach is essential when managing the gynaecological and obstetrical issues affecting women with FXIII deficiency.

7.2.2 Management of pregnancy and labour in FXIII deficient women.

Our study on normal pregnant women revealed FXIII level to be lower toward the 2^{nd} and 3^{rd} trimester of pregnancy compared to the 1^{st} trimester and control groups. The exact mechanism of such drop in FXIII activity during these stages of pregnancy is not clearly understood and need further study. Whether increased transplacental FXIII transport to the growing foetus and/or the increased FXIII consumption as part of placental growth are attributing to this decline in FXIII level require further studies. Similarly, it is also important to investigate whether plasma FXIII activity predict the presence of placental insufficiency or its related conditions such as intrauterine growth retardation and preeclampsia.

The decline in FXIII activity during immediate postpartum period was reported in this study when measured during day 0 to 3 post delivery. Future studies are required to define the time needed for FXIII level to return back to its pre-pregnancy level. This information can be useful in the clinical care of women with FXIII deficiency. Studies in pregnant women with FXIII deficiency are required to confirm whether the same pattern of change in their FXIII levels occur during pregnancy and postpartum as in the normal population.

7.2.3 FXIII level during menstrual cycle

FXIII activity was significantly low during menstrual and periovulatory phases compare to the last two weeks of the cycle. However, due to the weak association and the small number of women included in this study, it is still not certain whether such variation in FXIII level and the timing of blood sample taking during menstrual cycle can significantly affect the sensitivity of tests in diagnosis of FXIII deficiency.

Further future studies may be needed on women with FXIII deficiency to examine whether similar trend is also seen during menstrual and periovulatory phases of their cycle. Low FXIII levels during these periods, if similarly present in FXIII deficient women, can be related to the occurrence of menorrhagia and ovarian bleeding. If so, there might be a need to adjust the dose of FXIII prophylaxis during menstruation and ovulation periods.

7.2.4 Plasma FXIII activity and recurrent pregnancy loss

Although our study didn’t show a significant difference in mean FXIII activity in women with recurrent miscarriages when compared with the control group, there was a significantly higher number of women with FXIII <70 IU/dL among those with recurrent miscarriage. Whether this has a significant role in the pregnancy loss in such women need further studies. Large studies assessing FXIII levels in correlation with other haemostatic factors are required to assess whether a defect in haemostatic process presents a risk for early pregnancy loss or pregnancy related bleeding. The role of local endometrial haemostatic factors in the process of implantation and early placental development also require further research.

Finally, studies to assess the role of FXIII level systemically or locally within the endometrium/decidua in women undergoing in-vitro fertilisation and particularly those with recurrent implantation failure are also required.

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APPENDICES

Appendix 1

Summary of the case reports of women with congenital FXIII deficiency

Authors and year	**Fisher et al, 1966**	**Rodeghiero et al, 1987**(Rodeghiero et al., 1987)	**Burrows et al, 2000**(Burrows et al., 2000)
Country	Morocco	Italy	Australia
Age/age at Dx (year)	29/29	25/18	22/3
Family History	No	NS	brother FXIII <1U/dl. ICH at age 2.
Consanguineous	Yes	NS	NS
Abnormal tests	Abnormal clot solubility test	Abnormal clot solubility test. FXIII activity 0.5%. Sub unit B 45-60 U/dl	FXIII <1U/dl
Reason for Dx	bleeding diathesis & repeated miscarriage	NS	Repeated ICH at age of 3.
Treatment	Admitted 4 times for the pelvic hematoma Blood transfusion	Blood transfusion therapy post tooth extraction and retroperitoneal Hg.. External drainage and several Units of blood transfused to treat ICH, followed by 500U FFP every 3 weeks	Phenobarbital and carbamazepine for seizure
Regular prophylaxis	No	Substitutive therapy of 300ml-450ml FFP every 14 days for infertility. Became pregnant after 2 months One year later at age 21 regular 3 weekly FFP resumed in dose of 500U after ICH . While under prophylaxis became pregnant	250ml /mo pooled plasma. & prophylaxis 250-500IU FXIII concentrate
Infertility	NS	Yes with 2 years history of primary infertility	NS
Successful pregnancy	1	2	1
Miscarriage	12	Recurrent early miscarriages.Several onsets of unexplained delays in the onset of menstrual cycles. Pregnancy test was positive in 5-10 days followed by a heavy period	0
Prophylaxis during pregnancy	The patient was not on any prophylaxis for the last 12 pregnancies. For the last pregnancy, 300 ml plasma was given every 10days.	**1st pregnancy:** 300-450ml of FFP every 14 days. Mean pre-infusion FXII level was 2.5U/d.l Mean immediate post-infusion level 10 U/dl. **2nd pregnancy:** 500U FXIII concentrate every 3weeks.	FXIII concentrate increased to 500IU every 4weeks. Maintaining the level to 3 U/dl
Complications during pregnancy	Uneventful	NS	Uneventful
Prophylaxis for delivery	600ml plasma +1000ml fresh blood +300ml plasma 8 days postop	NS	1000IU FXIII was 19 U/dl
Mode of delivery & gestational age	Elective CS at 9th mo	**1st pregnancy** Emergency CS for cephalo-pelvic disproportion. **2nd pregnancy:** : NS	Induced NVD at 38wk,
Maternal outcome	Uneventful	Both pregnancies uneventful	Uneventful
Neonatal outcome	live baby 3000 gm,	Both pregnancies Full term healthy male baby	Healthy Boy wt. 3065 gm

Authors and year	**Ikkala et al, 1964(Ikkala et al., 1964)**	**Girolami et al 1986(Girolami et al., 1986)**	**Cerenzia et al, 1999(Cerenzia et al., 1999)**	**Takahashi et al 2007**
Country	Finland	Italy	Germany	Japan
Age/age at Dx	30/30	34/34	32/27	19/4
Family History	No	No	No	NS
Consanguineous	Yes	No	No	Ns
Abnormal tests	Abnormal clot solubility test	Abnormal TEG test and urea solubility test. FXIII<10U/dl	NS	FXIII activity after blood transfusion 7%. FXIII-A Ag <10%)
Reason for Dx	1962 severe intra-abdominal bleeding (ovarian origin)	Bleeding history since age of 8 following tonsillectomy and tooth extraction.PPH	Two attacks of ICH at age 15 and 17	At age of 4, subcutaneous haemorrhage on face with history of ICH at age 1 year
Treatment	Blood transfusion with removal of uterus and adenexia	NS	NS	200 ml Blood transfusion
Regular prophylaxis	Only after diagnosis prior to tooth extraction	NO	FXIII concentrate started 1990	Yes, FXIII concentrate
Infertility	NS	NS	NS	NS
Successful pregnancy	None	2	One (ended with IUD at 37 week of gestation with placental detachment)	One (2005)
Miscarriage	1959-1961 Repeated habitual	0	one at 6^{th} wk gestation (1991)	No
Prophylaxis during pregnancy	none	NO	FXIII concentrate (dose not specified)	500 U FXIII (Fibrogammin P) concentrate every 2-3 wks, maintaining FXIII level between 10-20%
Complications during pregnancy	NS	None	IUD at 37 week gestation	At 32 weeks of gestation, FXIII level dropped <3%. Fibrogammin increased to 1250 U /week and FXIII level raised to 10-20%
Prophylaxis for delivery	NA	None	3000 IU FXIII concentrate	1250 U Fibrogammin
Mode of delivery & gestational age	NA	NS	NVD at 37 week of gestation	NVD at 39 week
Maternal outcome	NA	PPH following both pregnancies	Uneventful	Uneventful
Neonatal outcome	NA	Two alive babies	IUD and delivery of dead baby 2600 gm	A live baby 2842 gm, FXIII activity was 21%. No bleeding.

Authors and year	**Boda et al, 1989**	**Kobayashi et al, 1990 Asahina et al, 1998 Asahina et al, 2000**
Country	Hungary	Japan
Age / age at Dx (years)	34/34	23/6
Family History	Her father's sister died of ICH. No other family had bleeding. Her brother is heterozygous	NS
Consanguineous	NS	NS
Abnormal tests	Plasma solubility in 5M urea <90%. FXIII – A subunit was not detected and B subunit was 25 U/dl. FXIII activity < 5%.	FXIII 7 U/dl
Reason for diagnosis	Preparation of operation to remove a breast tumour	Subdural haemorrhage at age of 6 years
Treatment	OCP for treating menorrhagia	Transfused fresh blood to treat umbilical and Subdural bleeding
Regular prophylaxis	FFP given prior to surgery in Dec 1986 for breast mass removal	NS
Infertility	Yes because she had history of primary infertility and had 8 subsequent miscarriages	NS
Successful pregnancy	1	2
Miscarriages	8	3
Prophylaxis during pregnancy	The first spontaneous miscarriages occur at 18th week of gestation. Other 7 miscarriages occurred between 8-12 wks . No major bleeding disorders 9th pregnancy: 400 ml FFP every 10 days after becoming spontaneously pregnant in March 1987	**1st pregnancy**: Spontaneous miscarriage at 2 month **2nd pregnancy**: In 1986, vaginal bleeding on 6w5d gestation,. Pre-infusion level of FXIII was 24 U/dl.. treated with 500IU /wk of FXIII concentrate **3rd pregnancy**: Spontaneous miscarriage at 2 month. **4th pregnancy**: At 5w6d had vaginal bleeding. mean pre-infusion level of plasma XIII was 18.5 U/dl. Two vials FXIII concentrate given and bleeding stopped **5th pregnancy**: pre-infusion level plasma XIII was 23U/dl at 8w6d. Two vials of prophylactic FXIII concentrate were administered at 5w6d. and7w0d. Missed receiving 2 substitute therapy at 7w6d and 8w6d.
Complications during pregnancy	NS	**2nd pregnancy**: 2 vial /wk of FXIII concentrate. From 9-22wk, plasma FXIII ranged from 12-30 U/dl. In 23rd wk, it reduced to 7%. From 24 to 37 wk, FXIII ranged from 10 -30 U/dl **4th pregnancy**: 1vial per wk started from the onset of bleeding, plasma FXIII ranged from 10-20 U/dl. At 23wk plasma FXIII dropped to 5 U/dl so the weekly dose increased to 2 vial per wk . and the plasma FXIII level was from 13-39 U/dl. No genital bleeding occurred. **5th pregnancy:** Fetal heartbeat disappeared at 9w5d).
Prophylaxis for delivery	400 ml FFP. Mean post infusion level of FXIII was 11 U/dl	**2nd pregnancy**: 4 vials of FXIII Concentrates. Patient FXIII level before delivery was 36 U/dl . **4th pregnancy:** 4 vials of FXIII Concentrates patient's plasma XIII before delivery was 52 U/dl.
Mode of delivery & gestational age	CS at 38 week	2nd and 4th pregnancy: Normal vaginal delivery on 37 wk
Maternal outcome	Uneventful	2nd and 4th pregnancy Uneventful with no bleeding
Neonatal outcome & its treatment	3100 gm Healthy boy. No sign of bleeding, FXIII activity was 45 U/dl	**2nd pregnancy**: Female infant weighed 2646 g and was in good condition. The plasma X III-Ag was 32 U/dl. **4th pregnancy:** Female infant weighed 3,142 g and was in good condition. The infant's plasma X III –Ag was 31 U/dl.

Authors and year	**Melo et al, 2008**	**Hanke et al , 2010**	**Rott et al, 2004**
Country	Colombia	Germany	Germany
Age/ age at Dx	19/19	32	35/ 35
Family History	two brothers died at 8 and 12 years of age .cousin FXIII deficiency at 12 yrs	Yes, her sister suffered from same disease	NS
Consanguineous	Yes	NS	NS
Abnormal tests	PT: 16, PTT 28 factor XIII activity was of 7.5 IU/dL	FXIII activity of 40% & Hereditary hypofibrinogenaemia Prolonged PT& TT, Normal aPTT & platelet	FXIII rest activity 50%. Genotype showed FXIII-B mutation
Reason for diagnosis	macroscopic Haematurea and profuse post sex bleeding	NS but the patient was reported for spinal anaesthesia during CS	Five unsuccessful IVF
Treatment	RBC transfusion post exodontias. tranexamic acid (1 gram /6 hours), 3 U FXIII concentrates of (1/20 kg), controlling bleeding. Haematoma treated with 7 U cryoprecipitate and tranexamic acid. Ovulation bleeding treated with 8 U cryoprecipitate preoperatively and 2 U packed RBC during operation	NS	The 6th IVF treated with 2500 IE FXIII-concentrate i.v. and she became pregnant.
Regular prophylaxis	Yes, cryoprecipitate monthly	NS	FXIII concentrate before the 6^{th} IVF
Infertility	No	NS	5 unsuccessful IVF
Successful preg	1	1	1
Miscarriages	No	NO	NS
Prophylaxis during pregnancy	cryoprecipitate 6 units monthly	NS	FXIII- concentrate substituted 2500 U every 10 days. FXIII activity was always above 70% during pregnancy. No signs of hypercoagulability occured, d-dimers and Prothrombin fragment normal.
Complications during pregnancy	Between weeks 29-34 had haematurea.	NO	Premature rupture of membrane on 38th week so she had CS
Prophylaxis for delivery	Week 39, 8 units of cryoprecipitate given prior to CS.	2gm fibrinogen & FXIII 1250IU . Preinfusion FXIII 48%. Post infusion FXIII 62%.	
Mode of delivery & gestational age	39week by CS	Elective CS for breech presentation at 38 week	CS on 38th week of gestation
Mat outcome	uneventful	uneventful	NS
Neonatal outcome	Normal test of FXIII	Alive baby	healthy, small for date. 2400 g wt.

Authors and year	**Gomez Garcia et al, 2001**	**Saito et al, 1990**
Country	Netherland	Japan
Age / age at diagnosis	1st case: NS/7 2nd case: NS/31	32/ 32
Family History	1st case: No 2nd case: Yes, her brother died age 12 from ICH	Yes, sister had two pregnancies with PPH at both times. Plasma FXIII activity <10U/dl Brother had undetectable FXIII-B. Plasma FXIII activity 10U/dl
Consanguineous	NS	Yes, parents are cousins
Abnormal tests	1st case: FXIII activity was 0.01 U/ml based on ammonia release assay 2nd case: FXIII activity was 0.02 U/ml based on ammonia release assay	Low FXIII level of activity (24U/dl) Undetectable subunit B (<2 U/dl) Reduced FXIII-A subunit (14 U/dl) Platelet FXIII A normal (135 U/dl)
Reason for diagnosis	1st case: long-lasting superficial bleeding after trauma. 2nd case: lifelong haemorrhagic diathesis, and after her brother's death due to ICH.	Bleeding at suture sites of 2nd CS.
Treatment	1st sister: Transfusion therapy for Posttraumatic intramuscular bleeding 2nd sister: FFP for intramuscular hematoma.	Suture bleeding treated with 5 units of concentrated Red cells and 5 units FFP
Regular prophylaxis	Since 1971, the sources of factor XIII were plasma, cryoprecipitate and, since 1992, plasma-derived factor XIII concentrate 2nd sister= NS	NS
Infertility	NS	No
Successful preg	1st case: 3 2nd case: 1	2
Miscarriage	1st Case: No 2nd Case: 10 spontaneous miscarriages	No
Prophylaxis in pregnancy	1st Case: FXIII substitutes 2nd case : NS	None
Complications during pregnancy	1st case: 2nd pregnancy: At 8th month developed APH. Other pregnancies had no bleeding 2nd case: all pregnancies had metrorrhagia at 4th month of pregnancy, except one at 7th month	1st pregnancy: uneventful 2nd pregnancy: vaginal bleeding near term
Prophylaxis for delivery	1st case: FXIII substitutes for all three pregnancies 2nd case: NS	None
Mode of delivery & gestational age	1st case: 2nd pregnancy at 8 month, mode not specified 2nd case: all terminated at 4 month except one at 7mo	1st pregnancy: CS for cephalopelvic disproportion 2nd pregnancy: CS
Maternal outcome & its treatment	NS	1st: uneventful 2nd: PPH bleeding treated with laprotomy, 5 U of concentrated RBCs and 5U FFP
Neonatal outcome	1st case: healthy 3 boys. All hetrozygose. FXIII activity 0.43 U/ml and 0.58 U/ml. Both sons had FXIII-A antigen reduce to 50% 2nd case: all terminated at 4 months except one pregnancy delivered a premature died in few days.	1st pregnancy: Live healthy girl. FXIII activity 107% 2nd pregnancy: full term healthy boy, FXIII activity 30%.

Authors and year	**Mikkola et al, 1997**	**Meili 2002**	**Singh et al 2008**	**Hamer and Rae 1971**
Country	Finland	Germany	India	New Zealand
Age/ age at Dx	1st sister: 38/19 2nd sister: 35/16	27/22	13/1	17/17
Family History	Yes, sister had bleeding symptoms	no	NS	Yes, one sister had umbilical cord bleeding and died age 2 due to ICH 2nd sister umbilical bleeding Mother bleed post labour
Consanguineous	NS	NS	NS	No
Abnormal tests	1st sister: Abnormal clot solubility no A-subunit antigen was detectable. FXIII activity 0.3%. 2nd sister: Abnormal clot solubility. FXIII activity 0.1%. Abnormal gene mutation	Abnormal clot solubility, FXIII activity <5% based on ammonia release assay	Normal PT, APTT	Abnormal clot solubility test
Reason for diagnosis	1st sister: younger sisters' bleeding symptoms. 2nd sister: In 1978, was investigated for haematuria, and intramuscular hematoma	corpus luteum rupture	Umbilical bleeding	History of bleeding diathesis
Treatment	Transfusion therapy for intramuscular bleeding 2nd sister: FFP for intramuscular hematoma.	Surgery to eliminate gluteal haematoma laprotomy for corpus luteum cyst rupture	surgery and 2 units of blood and 4 units of FFP.	Blood transfusion for surgeries. empiric treatment with FFP for trauma and dental treatment for 3 years
Regular prophylaxis	1st sister= No 2nd sister= NS	factor XIII concentrate (Fibrogammin) 500 U//month	FFP 15 mL kg/mo but only took it every 3 - 4 months. FFP/4–6 week post op	
Infertility	NS	NS	NS	NS
Successful preg	1st sister= 2; 2nd sister = 0	1	NS	NS
Miscarriage	No	NS	NS	NS
Prophylaxis during pregnancy	1st sister: 1st and 2nd pregnancy No blood products given	500 U Fibrogammin / 3 wks from 16th wks Increased to 750 U /2 wks from 22nd week	NS	NS
Complications during pregnancy	1st sister: 1st pregnancy: At 31 week had APH 2nd pregnancy: At 37 week had APH	NS	NS	NS
Prophylaxis for delivery	1st sister: 1st and 2nd pregnancies: FFP	1000 U Fibrogammin Keep FXIII activity 33U/dl	NS	NS
Mode of delivery & gestational age	1st sister: 1st pregnancy at 31 wk CS 2nd pregnancies at 37 wk CS	NVD at 37 week a healthy boy	NS	NS
Maternal outcome	NS	Normal	NS	NS
Neonatal outcome	Two healthy baby girl	healthy boy	NS	NS

Authors and year	**Padmanabhan et al, 2004**	**Chakravarty et al, 2012**
Country	India	India
Age / age at diagnosis	Case 1: 29/29 Case 2: 22/7	13/1
Family History	Case 1: NS Case 2: Yes, sister	NS
Consanguineous	Case 1: Yes, first cousins Case 2: Yes	NS
Abnormal tests	Case 1: Abnormal Urea solubility test Case 2: NS	NS
Reason for diagnosis	Case 1: Repeated seven first trimester miscarriages and bleeding diathesis Case 2: bleeding after tongue injury	umbilical cord bleed in the first week after birth, multiple bruises and hematoma.
Treatment	Case 1: Blood transfusion for tooth extraction Case 2: Blood transfusion for umbilical bleed	FFP. Ovarian haematoma (2L Blood) removed through laproscopy after 2 U FFP and 1 U blood prophylaxis. Intraop FFP, packed RB, and transexamic acid injection. Post op OCP.
Regular prophylaxis	Case 1: Cryoprecipitate, 1 unit every month for one year until got pregnant with the 8th pregnancy. repeated for 6 mo before 9th preg. Case 2: the 1st, 2nd, and 3rd pregnancies were not on prophylaxis. In the 4th pregnancy, cryoprecipitate infusion advised but not taken	FFP (15 ml/kg) per month But taken irregularly
Infertility	Case 1: NS Case 2: 2 maternal uncles with no children	NA
Successful pregn	Case 1: 1 Case 2: 0	NA
miscarriage	Case 1: 8 Case 2: 4	NS
Prophylaxis during pregnancy	Case 1: 8th pregnancy one units cryoprecipitate every 14 days. 9th pregnancy from 6 week gestation two units cryoprecipitate / 14 days. Case 2: None	NA
Complications during pregnancy	**Case 1**: 8th pregnancy: Missed miscarriage at 11wk . 9th pregnancy uneventful. **Case 2:** 1st:and 2nd pregnancy: spontaneous miscarriage in first trimester. 3rd pregnancy: Vaginal bleeding at 5wk. 4th pregnancy: vaginal bleed at 8 wk	NA
Prophylaxis for delivery	**Case 1**: 9th pregnancy: 2 units cryoprecipitate	NA
Mode of delivery	Case 1: 9th pregnancy: Induced NVD at 38wk	NA
Maternal outcome	Case 1: good	NA
Neonatal outcome	Case 1: 9th pregnancy alive baby Normal test of FXIII	NA

Authors and year	**Dargaud et al, 2008**	**Girolami et al, 1977**
Country	France	Italy
Age / age at diagnosis	34/34	**Case 1**: 30/ 28 **Case 2**: 33/33
Family History	NS	Yes (both cases were sisters)
Consanguineous	No	No
Abnormal tests	Normal: aPTT, Quick time, platelet, fibrinogen, VWF activity, plasma FXIII activity <15IU/dl. very low FXIII activity (2IU/dl). Ag measurement of FXIII-A and B subunits normal at 120 and 130IU/dl,	**Cases 1 and 2** Normal coagulation pattern. Electroimmuno assay showed lack of Subunit A, Normal subunit S.
Reason for diagnosis	Three spontaneous miscarriages.	**Case 1.** Evaluation of bleeding diathesis including APH, PPH. **Case 2** screening due to sister's bleeding symptoms.
Treatment	NS	No
Regular prophylaxis	NS	No
Infertility	NS	No
Successful pregnancy	1	**Case 1**: 2 **Case 2**: 2
miscarriage	3	**Case 1:** 2 miscarriage **Case 2:** No
Prophylaxis during pregnancy	Only for 4th pregnancy: FXIII concentrate 20U/kg every 21 days from the beginning of pregnancy	**Case 1**. No **Case 2:** No
Complications during pregnancy	**1st pregnancy** :miscarriage in 2002 at 8wk, associated with severe bleeding that required transfusion therapy **2nd pregnancy**: miscarriage in 2002 at 16wk. **3rd pregnancy:** VB after 4th wk, miscarriage in 2003 at 12 wks. **4th pregnancy:** at 12 wk, cervical insufficiency treated by Cervical cerclage. At 25 wk, Chorioamnionitis & PRM	**Case 1:** First pregnancy age 19 had APH and PPH Second pregnancy age 20, had APH, PPH Third pregnancy age 23, had miscarriage and profuse bleeding. Forth pregnancy age 25, had miscarriage and profuse bleeding. **Case 2:** both pregnancies had PPH
Prophylaxis for delivery	**4th pregnancy:** A booster dose FXIII concentrate 1250U	**Case1**: no Case 2: No
Mode of delivery	4th:Premature vaginal delivery at 25 wk	**Case 1**: NS
Maternal outcome & its treatment	3rd pregnancy: Post evacuation sever VB and DIC, treated with 10 units of FFP and 4 units red blood cells, and uterine artery embolisation controlled acute bleeding. 4th: Uneventful	**Case 1:** First pregnancy age 19 had APH and PPH treated with 3 unit blood transfusion with good response. Second pregnancy age 20, had APH, PPH and treated with blood transfusion Third pregnancy age 23, had miscarriage and profuse bleeding. Forth pregnancy age 25, had miscarriage and profuse bleeding. **Case 2:** Had one unit fresh blood for PPH.
Neonatal outcome	4th preg: premature, low birth wt 770 gm. Died 7 days after delivery due to ICH	NS

Authors and year	**Koseki et al, 2001**	**Lovejoy et al, 2006**	**Capellato et al, 1987**
Country	Japan	United States	Italy
Age / age at diagnosis	22/NS	NS	34/NS
Family History	NS	NS	NS
Consanguineous	NS	NS	NS
Abnormal tests	FXIII <10 IU/dL FXIII A <12 IU/dL using immunoassay	Undetectable A2B2 and FXIII-B subunit using urea clot solubility test	FXIII-A <10 IU/dL FXIII-B <10 IU/dL
Reason for diagnosis	Postoperative bleeding.	Long history of menorrhagia and postsurgical bleeding.	Subcutaneous and postoperative bleeding and menorrhagia
Treatment	NS	NS	NS
Regular prophylaxis	NS	NS	NS
Infertility	NS	NS	NS
Successful pregnancy	NS	NS	2
miscarriage	NS	NS	No
Prophylaxis during pregnancy	NS	NS	NS
Complications during pregnancy	NS	NS	Both pregnancies had PPH
Prophylaxis for delivery	NS	NS	NS
Mode of delivery	NS	NS	NS
Maternal outcome & its treatment	NS	NS	NS
Neonatal outcome	NS	NS	NS

Appendix 2

Characteristics of women in case series with congenital FXIII deficiency

	Burrow et al 2000	Lak et al 2003	Peyvandi et al 2004	Ivaskevicius et al 2007b	Ivaskevicius et al 2010b(Ivask	Ivaskevicius et al 2010a	Schroeder et al 2007	Vijapurkar et al 2009	Medhaffar et al 2006	Bhattchary et al 2005	Naderi et al 2012	Ichinose et al 2012
No.women	22	20	3	6	9 ††	8	5	2	4	3	17	7 ¶
Age	NS	NS	46,4 5,30	17-52	20-45	22-64	43,24 22,51 , 33	NS	NS	24, 29, 14	18-35	22-35
Age at Diagnosis	NS	NS	NS	13,2,34, 34,7, 20	NS	NS	5,0.5, 16,7, 22	NS	NS	24, 29,7	NS	NS
FamilyHx	NS	NS	NS	4/6	3/13	NS	1/5	NS	Yes	2/3	11/17	NS
Consang-uineous	3/16	NS	3/3	1/6	NS	0/8	NS	NS	NS	2/3	NS	NS
Ovulation bleeding	NS	4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NO
ICH	NS	NS	1/3	6/6	0/13	0/8	3/5	NS	NS	1/3	2/17	NO
Menorrhagia	I= 4/4 (3/3) * II=7/11 (3/5) III= 0/2	7/20 (35%)	3/3	1/6	1/13	1/8	NS	1/2	2/4	NS	NS	3/7
FXIII activity	NS	<5	18,6, <3	< 1.5	20-60	43-71	NS	< 3	<1	NS	NS	<2-10
Prophylaxis	NS	One case 8 U cryoppt	Only two cases	6/6 FXIII conc	2500 FXIII conc prior to CS	NS	NS	FFP	NS	One case FFP	FXIII conc	NS
RM **	Type I&III =0/6 TypeII= 10/16 (6/10) *	3/6 one case 13 RM then 2 preg after proph	One had 13 One had 5	NS	NS	NS	NS	NS	One case 5 RM	1st= 8 (RM) 2nd= 2 Miscarriage 3rd= 0	12/17 one misc 2/17 two misc 2/17 three misc	One had 2 miscarriage
No. Women had viable Pregnancy	I &III= 6/6(4/4) † II= 7/16 (2/10) *	6/20	NS	NS	1/9	2/8	NS	NS	0/4	NS	17/17 at 38 week	5/7
PPH	6/6(4/4†	NS	NS	NS	NO	2/8	NS	NS	NS	NS	NO	5/7

**RM=Recurrent Miscarriage NS= Not specified PPH = Post partum haemorrhage
* When excluding data from Ikkala et al, Fisher et al, Boda et al, Hamer and Rae et al, Kobayashi et al
† When excluding Saito et al and Girolami et al
†† Only 9/13 women were included with FXIII <70 IU/dL
¶ All the cases in Ichinosa paper were separately described in the case reports (Saito et al, Girolami et al 1977 and 1984, Lovejoy et al, Capellato et al, Koseki et al and Ivaskevicius 2010).

Appendix 3

History of bleeding diathesis among the studied case reports

	Umbilical bleeding	Intramuscular Haematoma	Haematurea	Post tooth extraction	Bleed after cut	Slow wound healing	ICH	Menorrhagia	Epistaxis	Bruising after trauma	Haemarthrosis	Post surgical bleed	Renal and GIT bleed	IintraAbdominal bleed corpus luteum rupture
Ikkala et al, 1964	+	-	-	-	-	-	-	+	-	-	+	-	-	+
Fisher et al, 1966	+	+	+	+	+	+	-	-	-	-	-	-	-	+
Hamer and Rae 1971	-	-	-	+	-	-	-	-	-	+	+	+	-	-
Girolami et al, 1977	- -	- -	- -	- -	- -	- -	- -	+ -	+ +	+ -	- -	+ +	- -	- -
Girolami et al, 1986	-	-	-	+	-	-	-	+	-	+	-	+	-	-
Capellato et al, 1987	-	-	-	-	-	-	-	+	-	+	-	+	-	-
Rodeghiero et al, 1987	+	-	-	+	-	+	+	+	-	-	-	-	-	-
Boda et al, 1989	+	+	-	-	-	-	-	+	+	-	-	-	-	-
Saito et al, 1990	-	-	-	-	-	-	-	-	-	+	-	+	-	-
Mikkola et al, 1997	- -	+ +	- +	- -	- -	- +	- -	- -	- -	+ -	- -	- +	- -	- -
Gerenzia et al, 1999	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Kobayashi et al, 1990 Asahina 1998, 2000	+	-	-	-	-	-	+	-	-	+	-	-	-	-
Burrows et al, 2000	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Gomez Garcia et al, 2001	- +	- +	- +	- +	- -	- -	- +	- +	- +	+ -	- -	- +	- -	- -
Meili 2002	+	+	-	+	+	+	-	-	+	-	-	+	-	+
Koseki et al, 2003	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Rott et al, 2004	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Padmanabhan et al, 2004	- +	- -	- -	+ -	+ +	- -	- -	- -	- -	- -	- -	- -	- -	- -
Lovejoy et al, 2006	-	-	-	-	-	-	-	+	-	-	-	+	-	-
Takahashi et al 2007	+	-	-	-	-	-	+	-	+	-	-	-	-	-
Dargaud et al, 2008	-	-	-	+	-	-	-	-	-	+	-	-	-	-
Melo et al, 2008	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Singh et al 2008	+	+	+	-	-	-	+	-	+	-	-	-	+	+
Hanke et al , 2010	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Chakravarty et al, 2012	+	+	-	-	-	-	+	-	-	+	-	-	+	+

Appendix 4

Pictorial Blood Assessment Chart and Scoring System for Assessment of Menstrual Blood Loss

How to use the PBAC scoring system:

- During the course of your period record your use of tampons and sanitary towels by placing a tally mark under the day next to the box that represents how stained your sanitary materials are each time you change them.
- Record clots by indicating whether they are the size of a 1p or 50p coin in the clots/ flooding row under the relevant day. E.g. under day 1 you may say 50p x 1 and 1p x 3.
- Record any incidences of flooding by placing a tally mark in the clots/ flooding row under the relevant day.

Scores:

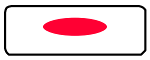
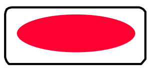
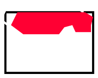
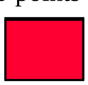
- A lightly stained towel (pic 1) will score 1 point, a moderately stained towel (pic 2) 5 points, a towel which is saturated with blood (pic 3) will score 20 points.
- A lightly stained tampon (pic 4) will score 1 point, a moderately stained tampon (pic 5) 5 points and a tampon that is fully saturated will score 10 points
- A clot the size of 1p scores 1 point, a 50p sized clot scores 5 points and flooding also scores 5 points

Results

Once you have finished your period total up your scores. A score of 100 or greater may indicate that you have heavy periods and you should seek advice from your doctor. However if your score is less than 100 and you have concerns about your period you should always consult your GP.

NAME: SCORE:
DAY START:

DAY

TOWEL	1	2	3	4	5	6	7	8
 1 point each								
5 points each 								
20 points each 								
CLOTS/ FLOODING Size 5 p = 1 point Size 50 p = 5 points								
TAMPON	1	2	3	4	5	6	7	8
1 point each 								
5 points each 								
10 points each 								
CLOTS/ FLOODING Size 5 p = 1 point Size 50 p = 5 points								

Higham et al, (1990), Assessment of menstrual blood loss using a pictorial chart, British Journal of Obstetrics & Gynaecology, 97, pp734-739.

Appendix 5 Assigned score for each bleeding symptom

Symptom	Score					
	−1	0	1	2	3	4
Epistaxis	–	No or trivial (less than 5)	>5 or more than 10′	Consultation only	Packing or cauterization or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Cutaneous	–	No or trivial (<1 cm)	>1 cm and no trauma	Consultation only		
Bleeding from minor wounds	–	No or trivial (less than 5)	>5 or more than 5′	Consultation only	Surgical haemostasis	Blood transfusion or replacement therapy or desmopressin
Oral cavity	–	No	Referred at least one	Consultation only	Surgical haemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Gastrointestinal bleeding	–	No	Associated with ulcer, portal hypertension, haemorrhoids, angiodysplasia	Spontaneous	Surgical hemostasis, blood transfusion, replacement therapy, desmopressin, antifibrinolytic	
Tooth extraction	No bleeding in at least two extraction	None done or no bleeding in one extraction	Referred in <25% of all procedures	Referred in >25% of all procedures, no intervention	Resuturing or packing	Blood transfusion or replacement therapy or desmopressin
Surgery	No bleeding in at least two surgeries	None done or no bleeding in one surgery	Referred in <25% of all surgeries	Referred in >25% of all procedures, no intervention	Surgical hemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Menorrhagia	–	No	Consultation only	Antifibrinolytics, pill use	Dilatation and currettage, iron therapy	Blood transfusion or replacement therapy or desmopressin or hysterectomy
Postpartum hemorrhage	No bleeding in at least two deliveries	No deliveries or no bleeding in one delivery	Consultation only	Dilatation and currettage, iron therapy, antifibrinolytics	Blood transfusion or replacement therapy or desmopressin	Hysterectomy
Muscle hematomas	–	Never	Post trauma no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Haemarthrosis	–	Never	Post trauma no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring Surgical intervention or blood transfusion
Central nervous system bleeding	–	Never	–	–	Subdural, any intervention	Intracerebral, any intervention